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## ERRATA

Page 151, line 25, "Sphaeropsideae" should read "Sphaerioidaceae."

Page 156, "*Penicillium camemberti*, var. *rogri*" should read "*Penicillium camemberti*, var. *rogeri*."

Page 296, Pl. XXXIII, figs. 3 to 15. The magnification of the illustrations should be half that stated in the legend.

Page 303, line 17, "Plate XXXVI, figures 1 to 4" should read "Plate XXXVII, figures 1 to 4" and "In figure 4, Plate XXXVI" should read "In figure 4, Plate XXXVII."

Page 318, Table IV, under head "General remarks," "rooting" should read "shooting."

Page 337, Table II, 4th column, "Phenolized defibrinated blood 3895 (unwashed)" should read "Phenolized defibrinated blood 3895."

Page 377, last line, 2d paragraph, "winged" should read "wingless."

Page 384, Table I, 6th column, "Current (milliampere minutes)" should read "Current (milliamperes)."

Page 388, line 13 from bottom, omit "with humidity at 57."

Page 419, line 25, "The twelve-spotted (or squash) lady beetle" should read "The squash lady beetle."

Page 419, line 28, "(*Crepidodera cucumeris*)" should read "(*Epitrix cucumeris*)."

Page 459, lines 2 and 24, omit "Three."

Page 471, line 4, "*Aleurodes mori* Ckll." should read "*Aleurodes mori*, var. *arizonensis* Ckll."

Page 762, Table I, first column, "*Medicago arbica*" should read "*Medicago arabica*."

Page 791, Table XIV, 1st column, "(p. 23)" should read "(p. 783)."

Page 865, legend under figure 5, "The solid black line, etc.," should read "The hatched line."

Page 866, legend under figure 6, omit sentences 2 and 3.

Page 881, line 6 from bottom, "April" should read "May."

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# RELATION OF CARBON BISULPHID TO SOIL ORGANISMS AND PLANT GROWTH[1]

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## INTRODUCTION

In a previous publication concerning the action of carbon bisulphid ($CS_2$) on bacteria and plants data were presented to show the beneficial effect of this substance on the soil flora (1).[2] The increased plant growth following the addition of carbon bisulphid in many cases is enormous. For example, a small application often causes an increase in yield from 100 to 200 per cent. It is impossible to account for this remarkable gain on the assumption that the only action of the carbon bisulphid is that of added plant food. It was found, as has been noted by many investigators (5, 6, 11, 12), that this volatile antiseptic exerts a very decided effect on the micro-organisms of the soil. As measured by plate counts, there is at first usually a great decrease in numbers, followed by a period of excessive increase, the total numbers far exceeding those that ordinarily exist. In certain cases carbon bisulphid has not only failed to cause an increase in plant growth, but has, on the contrary, caused a decrease.

Search has been made by many investigators for a satisfactory explanation of this peculiar action of carbon bisulphid. Many theories have been advanced. Concerning these theories so much has been written that a detailed discussion of the literature seems unnecessary. Indeed, it would be impossible within the limited scope of this paper to present a summary of the various explanations. One point is very prominent in nearly all of the publications: The action of carbon bisulphid is varied. Because of the interest attached to this problem, it was arranged to study some of the factors that might influence the action of carbon bisulphid. The experiments described in this paper are discussed under three main heads: First, the effect of varying amounts of carbon bisulphid; second, the effect of carbon bisulphid on various plants; and third, the effect of carbon bisulphid in various soils. In all of this work fresh field soil and commercial carbon bisulphid were used. Some of the experiments represent a combined study of the effect on both the lower and higher forms of plant life.

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station.
[2] Reference is made by number to "Literature cited," p. 18–19.

## EXPERIMENTAL METHODS

Commercial carbon bisulphid was poured into small holes in the soil, and these were covered immediately. The soil was sieved and potted in 2-gallon jars and the moisture maintained at half saturation. Changes in the soil flora were determined at regular intervals by plate counts of the number of bacteria and dilution counts of the number of active protozoa. The formation of ammonia and nitrates was measured at regular intervals.

The following plants were used: Buckwheat (*Fagopyrum fagopyrum*), clover (*Trifolium pratense*), corn (*Zea mays*), mustard (*Sinapis alba*), oats (*Avena sativa*), and rape (*Brassica napus*). In many of the experiments a first and a second crop were grown.

## EFFECT OF CARBON BISULPHID ON THE NUMBER AND ACTIVITY OF SOIL ORGANISMS

Eight jars were filled with Miami silt-loam soil from the Experiment Station farm. These were arranged in duplicate and treated as follows: (1) Control, untreated; (2) 2 per cent of carbon bisulphid; (3) 2 per cent of carbon bisulphid, evaporated; (4) 2 per cent of carbon bisulphid, evaporated, and reinoculated with 5 per cent of the original soil.

Twenty-four hours after treatment the soil in the evaporated series was spread out on sterile paper and the volatile antiseptic allowed to escape. At the end of the second 24-hour period the soil was put back into the jars. In order to prevent any contamination, the jars were covered with a double layer of cheesecloth and nonabsorbent cotton. This cover should allow free access of air without much danger of contamination. At regular intervals the covers were removed and samples drawn for analysis. The results of these determinations are presented in Tables I and II.

### NUMBER OF ORGANISMS

BACTERIA.—In Table I are shown the number of bacteria in 1 gm. of soil at different times and under the different conditions.

TABLE I.—*Effect of carbon bisulphid on number of bacteria*

| Time. | Bacteria per gram of dry soil. | | | |
|---|---|---|---|---|
| | Control. | 2 per cent of carbon bisulphid. | 2 per cent of carbon bisulphid evaporated. | 2 per cent of carbon bisulphid evaporated + 5 per cent of soil from control. |
| *Days.* | | | | |
| 1 | 11,496,000 | 1,965,000 | 2,260,000 | 2,358,000 |
| 3 | 22,010,000 | 23,975,000 | 8,254,000 | 12,480,000 |
| 5 | 20,635,000 | 25,253,000 | 27,416,000 | 95,499,000 |
| 9 | 14,739,000 | 36,651,000 | 61,904,000 | ..... |
| 13 | 16,115,000 | 90,473,000 | 98,850,000 | 80,420,000 |
| 21 | 19,508,000 | 60,149,000 | 71,257,000 | 52,495,000 |
| 25 | 18,272,000 | 68,276,000 | 86,483,000 | 64,570,000 |
| 29 | 15,346,000 | 90,645,000 | 84,272,000 | 38,495,000 |
| 60 | 12,372,000 | 58,101,000 | 60,000,000 | 30,000,000 |

At first the antiseptic causes a great reduction in the number of organisms capable of developing on Heyden agar. The period of depression lasts for only a short time—in this experiment about five days. From that time until the end of the test the number of organisms in the treated series far exceeded that of the control. The highest number in the carbon bisulphid evaporated and unevaporated soil occurred about the thirteenth day; while the carbon bisulphid evaporated soil plus control soil gave the highest count on the fifth day. At the time of the last count, 60 days after carbon bisulphid was added, the organisms in the treated series far exceeded those in the original soil. Apparently the effect of carbon bisulphid on the number of bacteria is noticeable for a long period of time.

If the results of the counts with carbon bisulphid unevaporated are compared with those of carbon bisulphid evaporated, it appears that no very marked difference exists. The greatest reduction in numbers occurred in soils with the carbon bisulphid evaporated. It is significant that soil with carbon bisulphid evaporated should prove more injurious to micro-organisms than the unevaporated. This agrees with Gainey (2, p. 592), who reports that the combined effect of the two processes seemed more injurious to nitrification than treatment with carbon bisulphid unevaporated.

After the thirteenth day the treated and reinoculated soil did not show as many organisms as the treated series. This difference is shown very distinctly in Plate I, which is reproduced from a photograph of a number of colonies developing on agar. Four parallel plates were made from the same dilution of each soil.

On this date samples were also drawn for ammonification tests. The purpose of this was to measure the rate of the decomposition of casein in the various series, and 1 per cent of casein was added to the soil and the ammonia determined after 12 and 24 hours. The beneficial effect of carbon bisulphid on ammonification is very evident. If after 12 hours the untreated is 100, then carbon bisulphid unevaporated is 154, carbon bisulphid evaporated is 212, and carbon bisulphid reinoculated is 190.

After 24 hours the untreated is equal to 100, carbon bisulphid unevaporated is 149, carbon bisulphid evaporated is 171, and carbon bisulphid reinoculated is 153. The data show very clearly that casein is decomposed more rapidly in treated than in untreated soils. This difference is most prominent in the 12-hour tests.

PROTOZOA.—Counts at the beginning showed the presence of protozoa in dilutions representing 1 to 1,000 gm. of soil (13, p. 626). Two weeks after treatment the soils were recounted. At this time numerous small flagellates were found in dilutions of 1 to 1,000. It is evident that the different treatments with carbon bisulphid had not seriously injured this group of organisms.

AZOTOBACTER.—One month after treatment with carbon bisulphid, qualitative tests were made. The Azotobacter organisms were found in all soils. The brown film of Azotobacter from the treated soils was not so profuse as that from the original soil.

ALGÆ.—In order to estimate the number of algæ, dilution tests were made. These cultures were incubated for 30 days. The smaller forms were found in great numbers in all of the soils.

The important facts in these data are (1) that the volatile antiseptic fails to remove these larger soil organisms and (2) that the smaller forms of bacteria are only temporarily reduced. The decrease in numbers is soon followed by a period of excessive growth.

## ACTIVITY OF ORGANISMS

A rapid multiplication of bacteria should naturally be followed by a parallel increase in decomposition products. Accordingly samples for analysis were drawn from the jars used in the previous experiment. The results of these periodic analyses are presented in Table II.

TABLE II.—*Effect of carbon bisulphid on ammonia and nitrate content of soil*

| Time. | Nitrogen per 100 gm. of dry soil. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ammonia. | | | | Nitrate. | | | |
| | Control. | 2 per cent of carbon bisulphid. | 2 per cent of carbon bisulphid evaporated. | 2 per cent of carbon bisulphid evaporated+5 per cent of soil from control. | Control. | 2 per cent of carbon bisulphid. | 2 per cent of carbon bisulphid evaporated. | 2 per cent of carbon bisulphid evaporated+5 per cent of soil from control. |
| *Days.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| At beginning | 1. 60 | 1. 60 | 1. 60 | 1. 60 | 2. 66 | 2. 66 | 2. 66 | 2. 66 |
| 30 | 1. 68 | 5. 27 | 5. 41 | 4. 71 | 3. 35 | 2. 50 | 2. 00 | 5. 55 |
| 45 | 2. 38 | 8. 40 | 7. 70 | 4. 90 | 3. 75 | 2. 70 | 2. 50 | 5. 66 |
| 60 | 2. 59 | 5. 43 | 5. 32 | 2. 31 | 4. 00 | 2. 81 | 2. 50 | 4. 50 |
| 75 | 1. 85 | 5. 60 | ..... | 2. 10 | 3. 20 | 2. 40 | 2. 60 | 5. 00 |
| 90 | 2. 94 | 4. 06 | 4. 06 | 2. 24 | 4. 00 | 5. 00 | 3. 32 | 6. 66 |

In the soils treated with carbon bisulphid there is a very decided accumulation of ammonia nitrogen. If the figures of Table I are compared with those of Table II, ammonia production, it will be seen that an increase in the number of bacteria within a certain range results in a gain in ammonia. After 30 days the amount of ammonia nitrogen in the treated soils averaged more than three times that in the original soil. After 60 days the ammonia content in the carbon bisulphid and carbon bisulphid evaporated soil was about double that of the control, while in the carbon bisulphid evaporated plus 5 per cent fresh soil it was

less. From the data it appears that reinoculation prevents large accumulations of ammonia. This is no doubt due to the oxidation of ammonia by the nitrifying bacteria. The figures of the last column (nitrate accumulation) support this statement. A stimulation of ammonification is still noticeable at the end of 3 months.

The nitrate-forming bacteria apparently do not recover so rapidly from carbon bisulphid treatment as the ammonia-producing organisms; consequently, there is no increase in nitrates until the end of 3 months. An exception to this is noted in the reinoculated soil. Here the activity of the nitrifying bacteria is evident 30 days after inoculation.

In order to ascertain, as nearly as possible, the effect of carbon bisulphid on the soluble nitrogen of the soil, the figures of Table II, ammonia and nitrate nitrogen, were combined in Table III.

TABLE III.—*Effect of carbon bisulphid on soluble nitrogen*

| Time. | Ammonia and nitrate nitrogen per 100 gm. of dry soil. | | | |
|---|---|---|---|---|
| | Control. | 2 per cent carbon bisulphid. | 2 per cent carbon bisulphid evaporated. | 2 per cent carbon bisulphid evaporated +5 per cent of soil from control. |
| *Days.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| At beginning | 4. 26 | 4. 26 | 4. 26 | 4. 26 |
| 30 | 5. 03 | 8. 47 | 7. 41 | 10. 26 |
| 45 | 5. 13 | 11. 10 | 10. 20 | 10. 56 |
| 60 | 6. 59 | 8. 24 | 7. 82 | 6. 87 |
| 75 | 5. 05 | 8. 00 | ..... | 7. 10 |
| 90 | 6. 94 | 9. 06 | 7. 38 | 8. 90 |

From the data in this table it is very evident that carbon bisulphid causes a large increase in ammonia and nitrate nitrogen. There seems to be very little difference between the effect of the various treatments of carbon bisulphid on the formation of ammonia and nitrate nitrogen. When compared with the control soil, it will be seen that 45 days after treatment the carbon-bisulphid soils contain more than twice as much soluble nitrogen. The higher ammonia and nitrate content is very marked 90 days after treatment. A repetition of this experiment gave similar results.

A review of the data in Tables II and III shows very clearly that carbon bisulphid in Miami soil increases the total soluble nitrogen—namely, ammonia and nitrates. One interesting fact that appears from a comparison of the ammonia and nitrate content is that these two substances are to a certain degree inversely proportional.

## EFFECT OF CARBON BISULPHID ON THE HIGHER AND LOWER FORMS OF PLANT LIFE

From the results of the preceding experiments it seems that carbon bisulphid should exert a beneficial effect on the growth of higher plants. At first this should be most marked with ammonia-feeding plants, and later with nitrate-feeding plants. Unfortunately it is not possible to secure plants that feed entirely on nitrates or ammonia. For this reason it was thought best to study the relation of carbon bisulphid to the growth of several different plants. Accordingly a combination study of the effect of carbon bisulphid on higher plants and on bacteria was made. A wide range of soil types, as well as different higher plants, was used.

Before entering upon a study of the relation of carbon bisulphid to soil type and various plants, it was desired to obtain some idea of the influence of various amounts of carbon bisulphid on plant growth. The procedure was as follows: Ten kgm. of field soil (Miami silt loam) were placed in each of sixteen 2-gallon jars. The carbon bisulphid was added in varying amounts, from 0.5 per cent to 2 per cent. It was poured into holes in the soil. These holes were closed immediately and the water increased to half saturation. In order to overcome the injurious effect of carbon bisulphid, the jars were then allowed to stand for two weeks before planting.

### CORN AND MUSTARD IN MIAMI SILT LOAM

The results of the test with corn and mustard are given in Table IV. It is evident from the data of the table that these plants do not respond alike to carbon bisulphid.

TABLE IV.—*Effect of varying amounts of carbon bisulphid on the growth of corn and mustard*

| No. | Soil. | Carbon bisulphid added. | Weight of corn. | | | Weight of mustard. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami. | Control. | 75 | 20 | 22.5 | 58 | 9.5 | 9.25 |
| 2 | ...do... | Control. | 80 | 25 | | 49 | 9 | |
| 3 | ...do... | 0.5 | 82 | 18 | 19.5 | 69 | 13 | 12.50 |
| 4 | ...do... | .5 | 83 | 21 | | 72 | 12 | |
| 5 | ...do... | 1 | 132 | 28 | 19.5 | 95 | 13 | 12.50 |
| 6 | ...do... | 1 | 22 | 11 | | 75 | 12 | |
| 7 | ...do... | 2 | 85 | 21 | 24 | 105 | 16 | 16.50 |
| 8 | ...do... | 2 | 125 | 27 | | 112 | 17 | |

In all concentrations except 2 per cent, carbon bisulphid injured the growth of corn. Mustard, on the other hand, was greatly benefited by the carbon-bisulphid treatment. An increased growth was observed from all concentrations. The maximum gain was noted with 2 per cent of carbon bisulphid. This beneficial effect on mustard is very evident from Plate II, figure 1. If this increase in growth is due to the larger

amount of soluble nitrogen as ammonia or nitrate, then corn and mustard should behave much alike. The nitrogen-feeding power of these plants has been studied by Krüger (8), Gerlach and Vogel (3), and others. It is supposed that both corn and mustard are heavy nitrogen-feeding crops, able to take nitrogen either in the form of ammonia or nitrate.

### BUCKWHEAT, CORN, AND OATS IN MIAMI SILT LOAM

In order to decrease the factor of individual variation, four parallel jars of Miami silt loam were used in each series in the following experiment. For the second crop these were subdivided into sets of two each. After the first crop was harvested, the soil and roots were thoroughly mixed and the jars replanted. The rotation was as follows: First crop, buckwheat; second crops, corn and mustard; first crop, corn; second crop, buckwheat; first crop, oats; second crops, corn and mustard. In Tables V, VI, and VII are presented the results of these experiments.

TABLE V.—*Effect of carbon bisulphid on the growth of buckwheat and corn*

| No. | Soil. | Carbon bisulphid added. | Weight of first crop, buckwheat. | | | Weight of second crop, corn. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami. | Control. | 90 | 15.5 | 19 | 152 | 28.5 | 31 |
| 2 | ...do... | Control. | 97 | 18 | | 160 | 33.5 | |
| 3 | ...do... | Control. | 121 | 22.2 | | ..... | ..... | |
| 4 | ...do... | Control. | 126 | 20.5 | | ..... | ..... | |
| 5 | ...do... | 2 | 124 | 23 | 24.5 | 169 | 34 | 32.7 |
| 6 | ...do... | 2 | 145 | 26.5 | | 136 | 31.5 | |
| 7 | ...do... | 2 | 127 | 23 | | ..... | ..... | |
| 8 | ...do... | 2 | 126 | 25.5 | | ..... | ..... | |

The yields of buckwheat and corn are given in Table V. The weights of the mustard were lost. Buckwheat gave an increase in the treated soil, while corn (the second crop) did not show any improvement. Determinations of ammonia present at the time the buckwheat was cut (three months after treatment) resulted as follows: Ammonia—if control is 100, then carbon bisulphid treated is 192. Nitrate—if control is 100, then carbon bisulphid treated is 28. The antiseptic increases ammonia, but decreases the nitrate content of soil. The results of investigation show that buckwheat feeds largely on nitrate nitrogen (9), while corn is supposed to be able to take its nitrogen in the form of ammonia. A difference in nitrogen-feeding power can not be used to explain the unequal behavior of these plants toward carbon bisulphid. Although the weights of the mustard crop were not kept, the action of the carbon bisulphid was evident. There was a decided gain in the growth of plants in the treated series.

From the data of Table VI it is obvious that carbon bisulphid has very little effect on corn (first crop) or buckwheat (second crop).

TABLE VI.—*Effect of carbon bisulphid on the growth of corn and buckwheat*

| No. | Soil. | Carbon bisulphid added. | Weight of first crop, corn. | | | Weight of second crop, buckwheat. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami | Control. | 480 | 85 | | 115 | 26 | |
| 2 | ...do... | Control. | 440 | 82 | 84.7 | 125 | 27 | 23.5 |
| 3 | ...do... | Control. | 500 | 90 | | 100 | 22 | |
| 4 | ...do... | Control. | 410 | 82 | | 95 | 19 | |
| 5 | ...do... | 2 | 380 | 77 | | 128 | 27 | |
| 6 | ...do... | 2 | 460 | 85 | 83 | 87 | 17 | 20.5 |
| 7 | ...do... | 2 | 410 | 83 | | 95 | 20 | |
| 8 | ...do... | 2 | 460 | 86 | | 87 | 18 | |

Table VII gives the effect of this volatile antiseptic on oats (first crop) and corn (second crop). The former showed an increase in growth in the treated soil; the latter was not affected.

TABLE VII.—*Effect of carbon bisulphid on the growth of oats and corn*

| No. | Soil. | Carbon bisulphid added. | Weight of first crop, oats. | | | Weight of second crop, corn. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami | Control. | 172 | 46.5 | | 166 | 40 | |
| 2 | ...do... | Control. | 184 | 51 | 48.3 | 132 | 31 | 36 |
| 3 | ...do... | Control. | 171 | 46.7 | | 118 | 29 | |
| 4 | ...do... | Control. | 182 | 49 | | 180 | 45 | |
| 5 | ...do... | 2 | 200 | 59 | | 155 | 37 | |
| 6 | ...do... | 2 | 205 | 59 | 57.8 | 161 | 38 | 37 |
| 7 | ...do... | 2 | 197 | 57.7 | | 152 | 37 | |
| 8 | ...do... | 2 | 192 | 57.5 | | 135 | 36 | |

A general consideration of the data shows that corn in this soil type is apparently indifferent toward carbon bisulphid. Buckwheat, oats, and mustard were all benefited by the antiseptic.

### BUCKWHEAT, MUSTARD, OATS, AND CORN IN DIFFERENT SOILS

The experiment with buckwheat, mustard, corn, and oats was a combination study of the effect of carbon bisulphid on bacterial activity and plant growth in three different soils. The first series contained Miami silt loam, the second series Miami soil diluted one-half by volume with sand, and the third series sand alone. According to chemical analysis, Miami silt loam is fairly rich in organic matter, nitrogen, potassium, and phosphorus. Of the three fertilizing elements, phosphorus perhaps is present in the smallest amount. The quantity of soil and its treatment was similar to that of the preceding experiment except that the treated jars were kept tightly covered with parchment paper. One month after the carbon bisulphid was added, these were removed. By

this means it was hoped to prevent a rapid volatilization of the antiseptic. The jars were not planted until three months after treatment.

At the beginning and at intervals of one, two, and three months bacterial activity was measured. Naturally, under the conditions of this experiment, carbon bisulphid proved very drastic. A great reduction in the number of bacteria, without any increase until the second month, was noted. The relation of carbon bisulphid to the number of bacteria was about the same in all three series. In the more compact type, Miami silt-loam soil, the carbon bisulphid proved most injurious to numbers, and consequently the period of increase was much later. Of the three soils, the treated sand showed the greatest proportional gain in number of bacteria.

Because of the severe nature of the carbon-bisulphid treatment, it was thought that probably the protozoa would be destroyed or the number greatly diminished. This was not the case, however, as protozoa were found in great numbers in both the treated and untreated soil.

Three months after treatment the jars were divided into two series and planted. The weights of the first and second crops are given in Tables VIII and IX.

TABLE VIII.—*Effect of carbon bisulphid on the growth of buckwheat and mustard in different types of soil*

| No. | Soil. | Carbon bisulphid added. | Weight of first crop, buckwheat. | | | Weight of second crop, mustard. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami silt loam..... | Control. | 123 | 22.5 | 21.5 | 12.0 | 3.4 | 3.3 |
| 2 | .....do..... | Control. | 107 | 20.5 | | 10.0 | 3.3 | |
| 3 | .....do..... | 2 | 119 | 25.0 | 24.0 | 24.5 | 5.2 | 7.3 |
| 4 | .....do..... | 2 | 114 | 23.0 | | 41.5 | 9.5 | |
| 5 | Half Miami silt loam, half sand. | Control. | 74 | 15.5 | 15.25 | 21.0 | 3.75 | 3.67 |
| 6 | .....do..... | Control. | 72 | 15.0 | | 17.0 | 3.60 | |
| 7 | .....do..... | 2 | 76 | 18.0 | 17.0 | 21.5 | 4.5 | 4.25 |
| 8 | .....do..... | 2 | 78 | 16.0 | | 17.0 | 4.0 | |
| 9 | Sand..... | Control. | 20 | 2.5 | 2.75 | 4.0 | 0.4 | .45 |
| 10 | .....do..... | Control. | 21 | 3.0 | | 4.5 | 0.5 | |
| 11 | .....do..... | 2 | 21.5 | 3.0 | 3.00 | 17.5 | 1.2 | .90 |
| 12 | .....do..... | 2 | 21.5 | 3.0 | | 5.5 | .6 | |

The figures of the buckwheat crop show the same general increase as noted in a previous experiment. Although not great, the gain in the treated series is consistent in all three soils.

The residual crop of mustard responded to a very marked degree to the carbon bisulphid treatment. In Miami silt loam the yield from the treated soil exceeded that of the control by more than 100 per cent. The gain in weight of oats in the treated soils was not so great, while the second-crop corn showed a loss (Table IX).

TABLE IX.—*Effect of carbon bisulphid on the growth of oats and corn in different types of soil*

| No. | Soil. | Carbon bisulphid added. | Weight of first crop, oats. | | | Weight of second crop, corn. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami silt loam..... | Control. | 162 | 47 | 47.7 | 114 | 25 | 24 |
| 2 | .....do..... | Control. | 180 | 48.5 | | 103 | 23 | |
| 3 | .....do..... | 2 | 157 | 45.5 | 49 | 77 | 18.5 | 19.2 |
| 4 | .....do..... | 2 | 190 | 52.5 | | 87 | 20 | |
| 5 | Half Miami silt loam, half sand..... | Control. | 82 | 26.5 | 27.2 | 76 | 16 | 15 |
| 6 | .....do..... | Control. | 85 | 28 | | 56 | 14 | |
| 7 | .....do..... | 2 | 85 | 27.5 | 28 | 52 | 13 | 14 |
| 8 | .....do..... | 2 | 82 | 28.5 | | 64 | 15 | |
| 9 | Sand..... | Control. | ..... | ..... | ..... | ..... | ..... | ..... |
| 10 | .....do..... | Control. | 18 | 6 | 6 | 12 | 4 | 4 |
| 11 | .....do..... | 2 | 18 | 5.8 | 5.5 | 14 | 5 | 4.5 |
| 12 | .....do..... | 2 | 15 | 5.2 | | 13.5 | 4 | |

The results of the nitrate determinations agree with those obtained in previous experiments. At the time of planting the carbon-bisulphid soils were lower in nitrate but higher in ammonia than the original soil.

The data from Tables VIII and IX show that carbon bisulphid has a much more beneficial effect on mustard than on any other crop. Buckwheat and oats are benefited, but not so markedly as mustard. Corn fails to show any improvement from treatment with carbon bisulphid.

## EFFECT OF CARBON BISULPHID ON BUCKWHEAT AND RAPE IN VARIOUS SOILS

The five soil types selected for the study of the effect of carbon bisulphid on buckwheat and rape in various soils ranged all the way from a very compact red clay to an open, sandy soil. After treating with 2 per cent of carbon bisulphid the soils were allowed to stand for three months before planting. Bacteria counts and nitrate determinations were made at the beginning and after two and three months. The effect of the carbon bisulphid on the total number of bacteria is very evident. In every case the carbon-bisulphid soil contained the most bacteria. The maximum gain occurred in the clay-loam soil, the minimum in the Norfolk sand. The increase due to the treatment was greatest after two months.

Here, again, the treated soils gave a much lower nitrate content than the controls. It seems safe to say that a rapid increase in numbers of bacteria in a carbon-bisulphid soil is followed by a decrease in the amount of nitrates.

Three months after treatment the soils were planted to buckwheat. Growth was slow at first, especially in the carbon-bisulphid series. The crop was harvested when 60 days old. The results of this experiment are shown in Table X.

TABLE X.—*Effect of carbon bisulphid on the growth of buckwheat in different types of soil*

| No. | Soil. | Carbon bisulphid added. | Weight of first crop. | | |
|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Cecil clay | Control. | 5 | 1.2 | 1.35 |
| 2 | .....do | Control. | 7 | 1.5 | |
| 3 | .....do | 2 | 12 | 3 | 3.5 |
| 4 | .....do | 2 | 15 | 4 | |
| 5 | Porters clay | Control. | 10.5 | 2 | 2.75 |
| 6 | .....do | Control. | 14.5 | 3.5 | |
| 7 | .....do | 2 | 14.5 | 3.7 | 3.15 |
| 8 | .....do | 2 | 10 | 2.7 | |
| 9 | Clay loam | Control. | 5.5 | 1.2 | 1.6 |
| 10 | .....do | Control. | 12.5 | 2 | |
| 11 | .....do | 2 | 28 | 6.5 | 7.25 |
| 12 | .....do | 2 | 30.5 | 8 | |
| 13 | Hagerstown loam | Control. | 27 | 6.2 | 5.6 |
| 14 | .....do | Control. | 25 | 5 | |
| 15 | .....do | 2 | 17.5 | 4.5 | 6 |
| 16 | .....do | 2 | 40.5 | 7.5 | |
| 17 | Norfolk sand | Control. | 32 | 8.7 | 9.45 |
| 18 | .....do | Control. | 49.5 | 10.2 | |
| 19 | .....do | 2 | 12 | 3 | 3.25 |
| 20 | .....do | 2 | 17.5 | 3.5 | |

With one exception, Norfolk sandy soil, the carbon-bisulphid series gave a larger yield. This was most marked in the case of clay-loam soil. The data on plant growth agreed with the plate counts.

The buckwheat was followed by a crop of Dwarf Essex rape. Unfortunately the young rape plants suffered seriously from insects. Although the tissue was too badly infested to save, a decided difference in growth could be seen. The beneficial effect of carbon bisulphid on rape was noted in every soil type.

### EFFECT OF CARBON BISULPHID ON VARIOUS CROPS IN ACID SOILS

In order to study the effect of carbon bisulphid on the growth of higher plants in acid soils, a series of experiments was made. Four types of soil were selected for this work: Miami silt loam, Sparta sand, Colby silt loam, and Marshfield peat. The neutral Miami silt loam was used as a check for the acid soils. According to the Truog acidity test, Sparta sand requires 0.5227 gm. of calcium carbonate per 100 gm. of soil, Colby silt loam 1.021 gm., and Marshfield peat 4.43 gm. Four weeks after treatment with carbon bisulphid, the soils were planted.

#### RED CLOVER

The effect of carbon bisulphid on medium red clover in acid soils is clearly seen from the figures of Table XI. The clover grew luxuriantly in all soils except the untreated acid peat. Two crops were cut. Carbon bisulphid in peat soil caused an enormous gain in the growth of clover. This was very striking in both the first and second crop.

TABLE XI.—*Effect of carbon bisulphid on the growth of red clover in acid soils*

| No. | Soil. | Carbon bisulphid added. | Weight of first crop, clover. | | | Weight of second crop, clover. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average green. | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami silt loam | Control. | 138 | | 139 | 129 | 19 | 20 |
| 2 | do | Control. | 140 | (a) | | 145 | 21 | |
| 3 | do | 2 | 158 | | 141 | 168 | 26 | 23 |
| 4 | do | 2 | 124 | | | 131 | 20 | |
| 5 | Sparta sand | Control. | 36 | | 34 | 58 | 13 | 11 |
| 6 | do | Control. | 33 | (a) | | 48 | 10 | |
| 7 | do | 2 | 19 | | 25 | 18 | 4 | 6 |
| 8 | do | 2 | 31 | | | 43 | 8 | |
| 9 | Colby silt | Control. | 95 | | 91 | 110 | 20 | 17 |
| 10 | do | Control. | 87 | (a) | | 85 | 14 | |
| 11 | do | 2 | 153 | | 143 | 108 | 15 | 13 |
| 12 | do | 2 | 133 | | | 82 | 12 | |
| 13 | Peat | Control. | 4 | | 3 | 6 | 2.8 | 2.4 |
| 14 | do | Control. | 2 | (a) | | 5 | 2 | |
| 15 | do | 2 | 83 | | 81 | 53 | 9 | 8.7 |
| 16 | do | 2 | 79 | | | 46 | 8.5 | |

[a] Lost.

Plate II, figure 2, shows the relative growth of clover in the treated and untreated soils.

Each figure for Miami silt loam in Table XI represents the average of triplicate jars. Because of the individual variation, it was decided to use 12 jars for this experiment. Six of these were used as controls and six treated with 2 per cent of carbon bisulphid. It is evident from the data that medium red clover in Miami soil is benefited both in the first and second crop by the antiseptic. In the Sparta sand a decrease was noted with each crop. The Colby silt loam gave a decided increase with the first crop, but not with the second.

Previous tests with these soils showed that the clover bacteria were present in sufficient numbers to produce good inoculation. In view of the large amount of carbon bisulphid applied, it was thought that this substance would probably injure nodule formation. However, examination of the root systems showed this was not the case. The plant roots were thoroughly inoculated, both in the treated and untreated soils. Apparently the plants in carbon bisulphid soils contained the greater number of nodules.

Because of the remarkable action of carbon bisulphid in peat soil, this part of the previous test was repeated. In addition to carbon bisulphid, the effect of flowers of sulphur was studied. If the data in the previous experiment are correct, the carbon bisulphid should greatly increase the growth of clover. A glance at the results in Table XII confirms this statement.

TABLE XII.—*Effect of carbon bisulphid and sulphur on the growth of red clover in peat soil*

| No. | Soil. | Treatment. | Weight. | | |
|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. |
| | | | *Gm.* | *Gm.* | *Gm.* |
| 1 | Peat | Control | 34 | 9 | 8.6 |
| 2 | do | do | 30 | 8.2 | |
| 3 | do | 1 per cent of carbon bisulphid. | 95 | 21.5 | 21.7 |
| 4 | do | do | 110 | 22 | |
| 5 | do | 2 per cent of carbon bisulphid. | 195 | 23 | 21.2 |
| 6 | do | do | 90 | 19.5 | |
| 7 | do | 0.3 per cent of sulphur. | 8 | 3.5 | 2.2 |
| 8 | do | do | 4 | 1 | |

Carbon bisulphid causes a remarkable increase in the growth of clover on peat soil. There is apparently no decided difference in the action of 1 or 2 per cent of carbon bisulphid. Just why the volatile antiseptic should stimulate so markedly the growth of clover in the peat soil is not known. A more detailed study of the action of carbon bisulphid in peat is now under way. Flowers of sulphur at the rate of 0.3 per cent proved very injurious. In view of the high sulphur content of carbon bisulphid, it was thought that possibly free sulphur in peat might have somewhat the same effect.

### CORN AND MUSTARD

The action of carbon bisulphid on corn and mustard in acid soils was studied in an experiment the results of which are given in Table XIII.

TABLE XIII.—*Effect of carbon bisulphid on the growth of corn and mustard in acid soils*

| No. | Soil. | Carbon bisulphid added. | Weight of corn. | | | Weight of mustard. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami silt loam | Control. | 190 | 50 | 64.5 | 83 | 18 | 19.5 |
| 2 | do | Control. | 360 | 79 | | 145 | 21 | |
| 3 | do | 2 | 315 | 70 | 65 | 159 | 27 | 25.5 |
| 4 | do | 2 | 320 | 60 | | 150 | 24 | |
| 5 | Sparta sand | Control. | 65 | 17 | 17.5 | 13 | 2.5 | 3 |
| 6 | do | Control. | 70 | 18 | | 19 | 3.5 | |
| 7 | do | 2 | 100 | 21 | 23.5 | 11 | 2 | 2.5 |
| 8 | do | 2 | 120 | 26 | | 12.5 | 3 | |
| 9 | Colby silt | Control. | ..... | 83 | 84.5 | ..... | ..... | 10 |
| 10 | do | Control. | 393 | 86 | | 67 | 10 | |
| 11 | do | 2 | 390 | 85 | 81 | 62 | 9.6 | 9.6 |
| 12 | do | 2 | 385 | 77 | | ..... | ..... | |
| 13 | Peat | Control. | 160 | 28 | 24 | 0 | ..... | ..... |
| 14 | do | Control. | 130 | 20 | | 0 | ..... | ..... |
| 15 | do | 2 | 165 | 25 | 22.5 | 0 | ..... | ..... |
| 16 | do | 2 | ..... | 20 | | 0 | ..... | ..... |

It is clear from the data that carbon bisulphid does not materially benefit corn. An exception to this was seen in the case of Sparta sand; in this instance the treated series showed a slight improvement.

A comparison of the growth of mustard in acid and in neutral soil shows that this crop grows best in a neutral soil. In Sparta sand and Colby silt loam the yield of mustard in the treated soil was below that of the control, while in the peat soil it failed entirely. It seems very probable that the acid reaction of the soil inhibits the growth of mustard. For instance, Kossovich (7) reports that mustard is sensitive to acidity. The addition of 2 per cent of carbon bisulphid to Miami soil stimulated the growth of mustard. This agrees with the results of previous tests. An increase in the growth of mustard has been noted in all four experiments with carbon bisulphid in Miami soil.

One series of jars, corn on Miami silt loam, was replanted to buckwheat. As previously reported, buckwheat showed a distinct improvement in the carbon-bisulphid soil. If the control weights are taken as 100, the treated series is equal to 115.

A review of all the data on the effect of carbon bisulphid on higher plants shows very clearly that carbon bisulphid does not produce the same effect on all plants. In almost every case (except acid soils) the carbon bisulphid favors in a decisive way the growth of mustard. Next in order of their response to carbon bisulphid come rape, red clover, buckwheat, oats, and corn. In acid soils, especially those rich in organic matter, the growth of clover is greatly favored by the carbon-bisulphid treatment.

The majority of the evidence indicates that carbon bisulphid is most beneficial to the growth of higher plants in peat or in open, sandy soils.

## EFFECT OF CARBON BISULPHID ON THE GROWTH OF PLANTS IN SILICA SAND

If carbon bisulphid is a plant stimulant, then the addition of the proper amount to a nutrient solution for plants should exert a beneficial effect on the growth of higher plants. To test this a series of experiments was performed on different plants.

### BUCKWHEAT AND OATS

Eight jars were filled with pure silica sand (99 per cent pure quartz), and the following ingredients added to each jar:

| | |
|---|---|
| Water ($H_2O$) | 500 c.c. |
| Potassium nitrate ($KNO_3$) | 5 gm. |
| Ferrous phosphate ($Fe_3(PO_4)_2$) | 1.25 gm. |
| Calcium phosphate ($Ca_3(PO_4)_2$) | 1.25 gm. |
| Calcium sulphate ($CaSO_4$) | 1.25 gm. |
| Magnesium sulphate ($MgSO_4$) | 1.25 gm. |

In addition to the soluble plant food, half of the jars received 2 per cent of carbon bisulphid. After treatment the jars were held for two months before planting to buckwheat and oats. The results of the test are given in Table XIV.

TABLE XIV.—*Effect of carbon bisulphid on the growth of buckwheat and oats in silica sand*

| No. | Carbon bisulphid added. | Weight of buckwheat. | | | Weight of oats. | | |
|---|---|---|---|---|---|---|---|
| | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Control. | 15.8 | 3.2 | 2.3 | 3.5 | 1.5 | 1.3 |
| 2 | Control. | 8.5 | 1.4 | | 3.4 | 1.2 | |
| 3 | 2 | 37.5 | 7 | 5.6 | ..... | ..... | 6.5 |
| 4 | 2 | 21 | 4.2 | | 21 | 6.5 | |

It is apparent from the data that carbon bisulphid in silica sand exerts a beneficial effect on the growth of both buckwheat and oats. This agrees with the results of Koch (6)—that carbon bisulphid stimulates the higher plant growth. Although the duplicate jars do not agree very closely, the highest yield of the control was lower than any of the treated groups. For some unexplainable reason, the oats in jar 3 failed to grow. The young seedling died soon after germination. Plate II, figure 3, is a reproduction of a photograph of the buckwheat series.

### CLOVER, BUCKWHEAT, AND MUSTARD

The foregoing experiment was repeated, using 3-kgm. jars and Tollen's medium. Only 1 per cent of carbon bisulphid was added. The jars were planted 30 days after treatment. The yields of the different crops are presented in Table XV. From the beginning clover and mustard began to show the favorable effect of carbon bisulphid.

TABLE XV.—*Effect of carbon bisulphid on the growth of buckwheat, clover, and mustard in silica sand*

| No. | Carbon bisulphid added. | Weight of buckwheat. | | | Weight of clover. | | | Weight of mustard. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Green. | Dry. | Average. | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Control. | 49 | 7.5 | 7 | 4.5 | 1 | 1.4 | 77 | 9 | 9 |
| 2 | Control. | 41 | 6.6 | | 10 | 1.8 | | ..... | ..... | |
| 3 | 1 | 49 | 7.8 | 7.6 | 12 | 2.2 | 2.2 | 52 | 5.8 | 8.6 |
| 4 | 1 | 45 | 7.5 | | 12 | 2.3 | | 98 | 11.5 | |

As compared with the results shown in Table XIV, the increase in the growth of buckwheat with carbon bisulphid was much smaller. The clover crop was about doubled in the presence of carbon bisulphid. Mus-

tard did not do well in sand cultures; growth was very irregular. Because of the size of the jars and the irregular growth of the crops it will be necessary to repeat the experiment.

### EFFECT OF CARBON BISULPHID IN REINOCULATED SOIL

In the first part of this paper it has been shown that if soil treated with carbon bisulphid is reinoculated with fresh soil the bacterial processes are altered. The increase in number of bacteria attains a maximum much sooner and begins to decline earlier than in soil treated with carbon bisulphid but not reinoculated. This is also noted in the formation of soluble nitrogen. In order to record the effect on plant growth, the following experiment was planned. Six jars with 9 kgm. each of Miami silt-loam soil were used. Two months after treatment with carbon bisulphid, 2 per cent of untreated soil were added to jars 5 and 6. An equal amount was removed before the original soil was added. All of the jars were kept for another month before planting.

Plate counts three months from the date of treatment showed a decided increase in number of bacteria in the carbon-bisulphid soils. No appreciable difference existed between the carbon bisulphid and the carbon-bisulphid reinoculated soil.

The effect of treatment on nitrate content is evident from the following figures: If the nitrate nitrogen at the beginning is 100, then the control after three months is 370, carbon bisulphid is 50, and carbon bisulphid plus 2 per cent of the original soil is 44. Here, again, the inverse relation of number of bacteria and nitrate content is noted.

Protozoa were found in all of the soils and apparently in about the same number two months after treatment as in the original soil.

The effect of this treatment on the growth of oats and corn may be seen from the figures in Table XVI.

TABLE XVI.—*Effect of carbon bisulphid on the growth of oats and corn in reinoculated soil*

| No. | Soil. | Treatment. | Weight of first crop, oats. | | | Weight of second crop, corn. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Green. | Dry. | Average. | Green. | Dry. | Average. |
| | | | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Miami. | Control | 168 | 51 | 50.9 | 107 | 28 | 29 |
| 2 | ...do... | .....do | 178 | 50.75 | | 108 | 30 | |
| 3 | ...do... | 2 per cent of carbon bisulphid. | 178 | 50 | 52 | 86 | 33 | 29.5 |
| 4 | ...do... | .....do | 185 | 54 | | 83 | 26 | |
| 5 | ...do... | 2 per cent carbon bisulphid plus 2 per cent of the original soil. | 178 | 51.5 | 56.5 | 79 | 22 | 24 |
| 6 | ...do... | .....do | 215 | 61.5 | | 101 | 26 | |

The average dry weight of oats in soil treated with carbon bisulphid was slightly greater than that of the control. This difference was most noticeable in the case of reinoculated soil. It appears that the reinoculation benefits the action of carbon bisulphid on the growth of oats. The second crop of corn gave the opposite results. The corn in untreated soil gave the highest yield.

## EFFECT OF CARBON BISULPHID ON THE ACCUMULATION OF SULPHATES IN SOIL

Very soon after the jars were planted it was observed that the surface of carbon-bisulphid soil was partly covered with needle-like crystals. Qualitative tests showed that these were made up largely of sulphates, possibly magnesium sulphate. The occurrence of salts was noted in several of the soils treated with carbon bisulphid. Possibly a part of the carbon bisulphid was oxidized to sulphates. It has been reported that a small portion of the carbon bisulphid may be converted into sulphates (4, p. 247–251; 10, p. 151–152).

Samples of the treated and untreated soils were analyzed for sulphates.[1] The results are shown in Table XVII.

TABLE XVII.—*Effect of carbon bisulphid on the accumulation of sulphates in the soil*

| No. | Time. | Treatment. | Sulphur as sulphates. |
|---|---|---|---|
| | *Months.* | | *Per cent.* |
| 1 | 1 | Untreated | 0.023 |
| 2 | 1 | 2 per cent of carbon bisulphid | .038 |
| 3 | 3 | Untreated | .018 |
| 4 | 3 | 2 per cent of carbon bisulphid | .039 |
| 5 | 5 | Untreated | .019 |
| 6 | 5 | 2 per cent of carbon bisulphid | .060 |

It is apparent from the data in this table that the addition of carbon bisulphid tends to increase the sulphate content of the soil.

## CONCLUSIONS

The addition of carbon bisulphid to soil exerts a decided effect on the fauna and flora of the soil. This is characterized by a temporary reduction in the number of micro-organisms. Later, an enormous multiplication of bacteria takes place and an almost parallel increase in production of by-products or soluble nitrogen is noted. The ammonia content seems to follow the curve of bacterial growth and later gives way to larger amounts of nitrate. From the evidence it seems that carbon bisulphid in soil produces an increase in soluble compounds of nitrogen and sulphur.

[1] The author is indebted to Prof. W. E. Tottingham, of the Department of Agricultural Chemistry, for the analyses.

In Miami soil carbon bisulphid benefited the growth of buckwheat, oats, and mustard. No relation seems to exist between plant stimulation with carbon bisulphid and the form of the soluble nitrogen. In non-acid soils carbon bisulphid is most beneficial to sulphur crops. Mustard offers a good example. In all of the experiments, except acid soils, mustard showed an increased growth from the use of carbon bisulphid. Carbon bisulphid in peat soil greatly benefits the growth of red clover. In sand cultures plus soluble plant food carbon bisulphid favors the growth of certain plants.

The data show clearly that carbon bisulphid does not act alike in all soils or toward all crops.

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## PLATE I

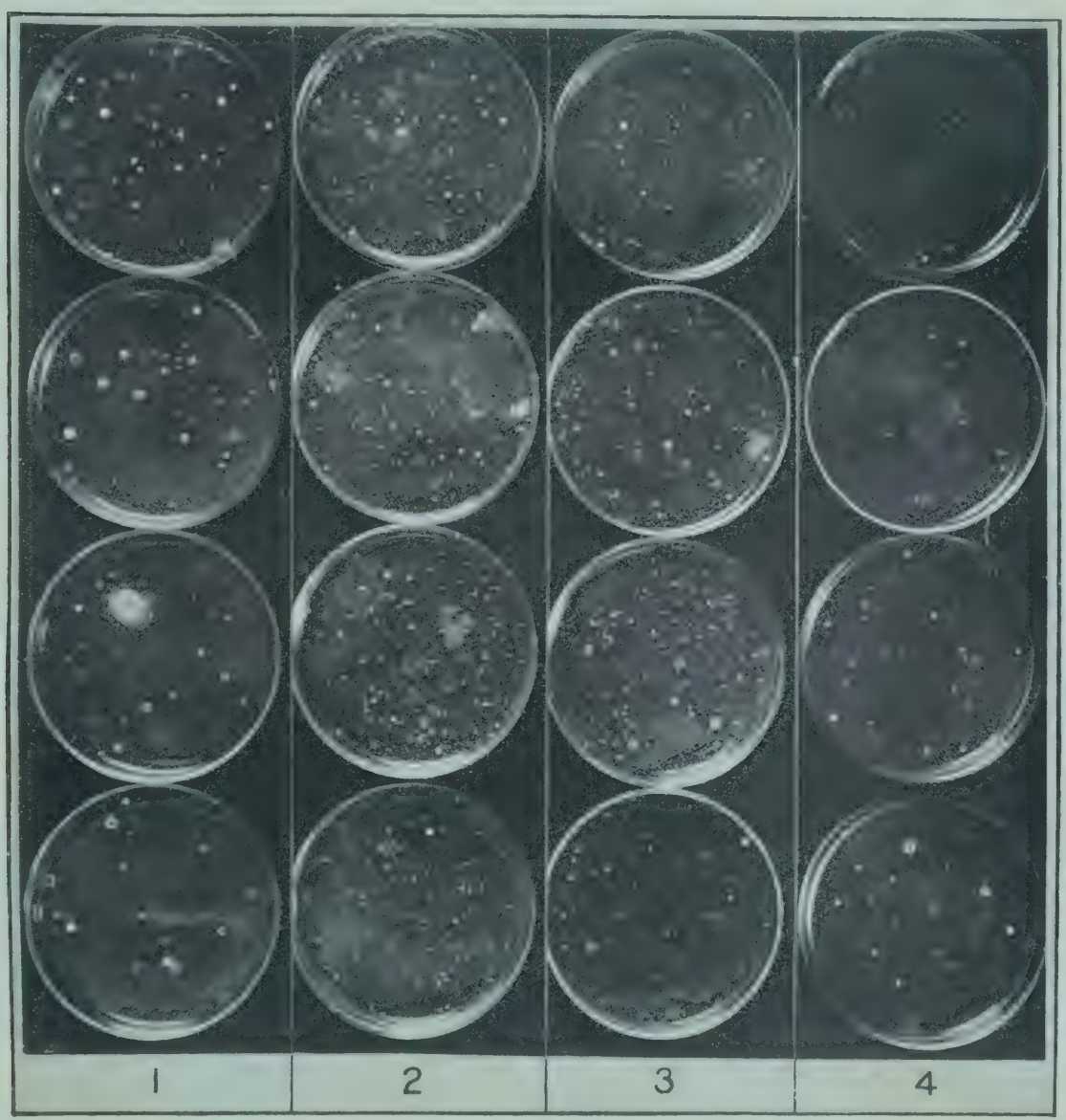
Plate cultures of soil organisms growing on agar:

Fig. 1.—Colonies of organisms from untreated soil.

Fig. 2.—Colonies from soil treated with 2 per cent of carbon bisulphid.

Fig. 3.—Colonies from soil treated with 2 per cent of carbon bisulphid and evaporated.

Fig. 4.—Colonies from soil treated with 2 per cent of carbon bisulphid, evaporated, and reinoculated with 5 per cent of soil from an untreated jar.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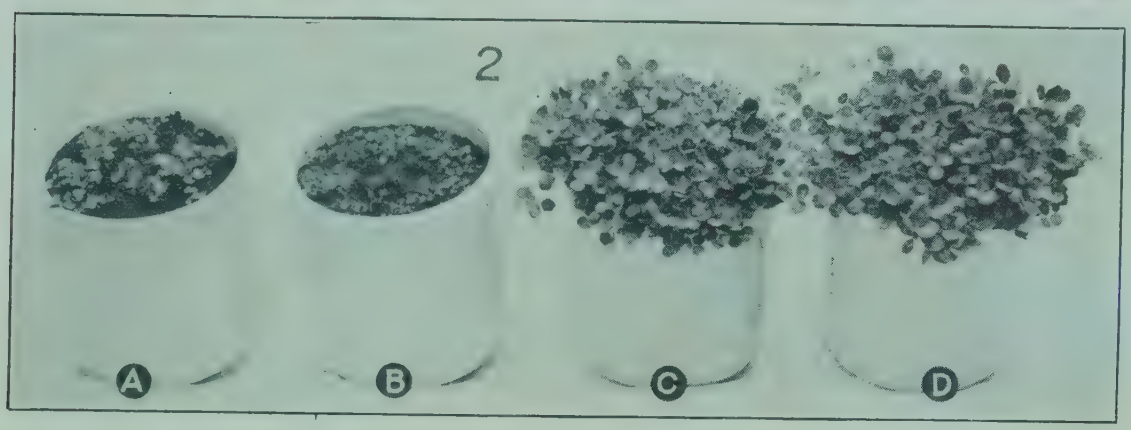
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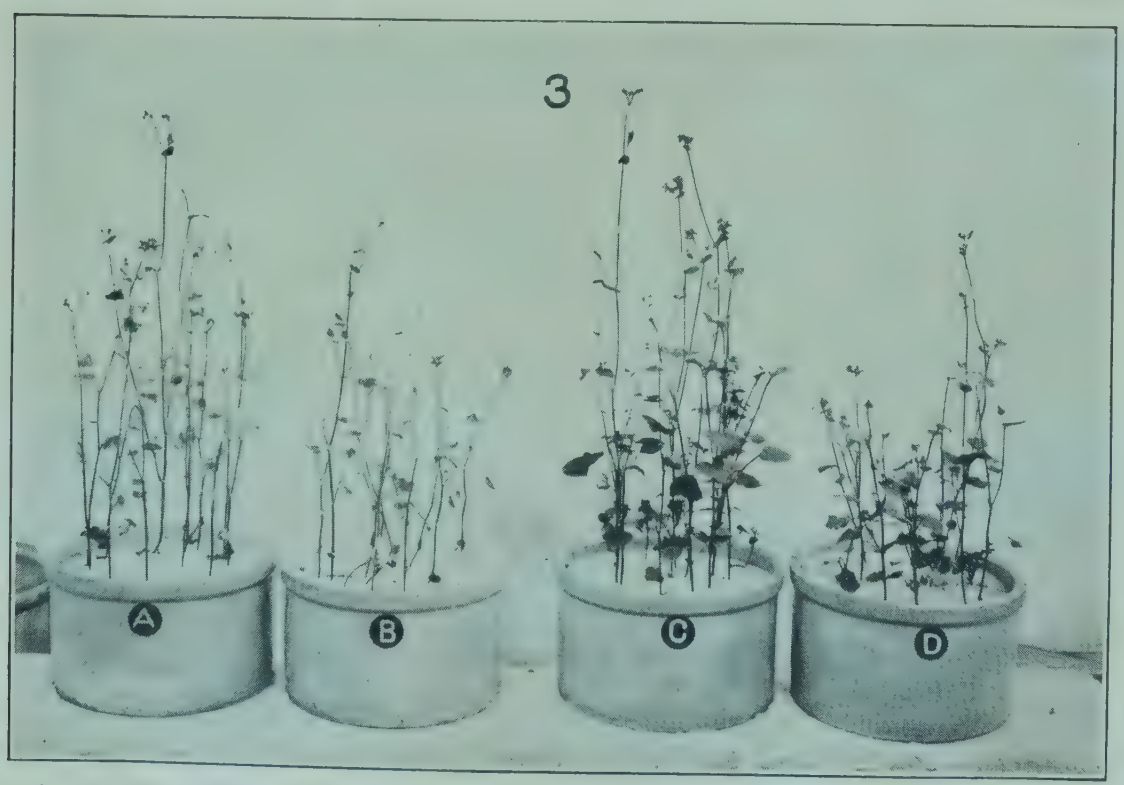
3
A
B
C
D

## PLATE II

Fig. 1.—Effect of varying amounts of carbon bisulphid on mustard; *A*, *B*, soil untreated; *C*, *D*, soil treated with 0.5 per cent of carbon bisulphid; *E*, *F*, soil treated with 1 per cent of carbon bisulphid; *G*, *H*, soil treated with 2 per cent of carbon bisulphid.

Fig. 2.—Effect of carbon bisulphid on clover in peat soil; *A*, *B*, soil untreated; *C*, *D*, soil treated with 2 per cent of carbon bisulphid.

Fig. 3.—Effect of carbon bisulphid on buckwheat in sand cultures; *A*, *B*, soil untreated; *C*, *D*, soil treated with 2 per cent of carbon bisulphid.

# CLIMATIC CONDITIONS AS RELATED TO CERCOSPORA BETICOLA [1]

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## INTRODUCTION [2]

Climatic conditions of both winter and summer bear an important relation to the vitality and development of *Cercospora beticola*. During cold weather certain conditions enable the fungus to overwinter, while certain other conditions are inimical to its growth, a fact which has an important bearing on the control of the disease, as the earliest infections on growing sugar beets (*Beta vulgaris*) originate from the overwintered fungus. In the early summer, after infection occurs, temperature, relative humidity, rainfall, and wind directly affect the development of the fungus, the rapidity of conidial production, and subsequent infection.

## OVERWINTERING

From the investigations here described it seems evident that under ordinary field conditions of winter the conidia of *C. beticola* usually live but a short time, although under ordinary herbarium conditions desiccation takes place only after exposure for several months. The sclerotia-like bodies (fig. 1, *A*, *a*), or masses of mycelium, the most resistant part of the fungus, which are embedded in the infected areas of the leaf blades and petioles, however, live over the winter under favorable conditions and in the spring produce conidia from the remnants of the old conidiophores (fig. 1, *A*, *b*), or both conidiophores and conidia (fig. 1, *A*, *c*) may be formed anew. For the purpose of making direct microscopical observation of such development sections of infected tissue which had been stored throughout the winter under favorable conditions were placed in hanging-drop cultures of bean agar. New conidiophores (fig. 1, *B*, *b*) grew from the masses of embedded mycelium, and although somewhat abnormal they produced rather typical conidia (fig. 1, *B*, *c*), thus showing that such material may be a source of early infection of growing plants.

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[1] The investigations were carried on entirely in the field. Preliminary work was conducted during 1911 and 1912 at Rocky Ford, Colo. The detailed data were collected during 1912 and 1913 at Rocky Ford, which is in the Arkansas Valley of Colorado, a semiarid region under irrigation, and during 1914 near Madison, Wis., where the rainfall and average humidity were greater.

[2] The writers are indebted to Mrs. Nellie E. Fealy, of the Bureau of Plant Industry, for aid in editing and revising the manuscript.

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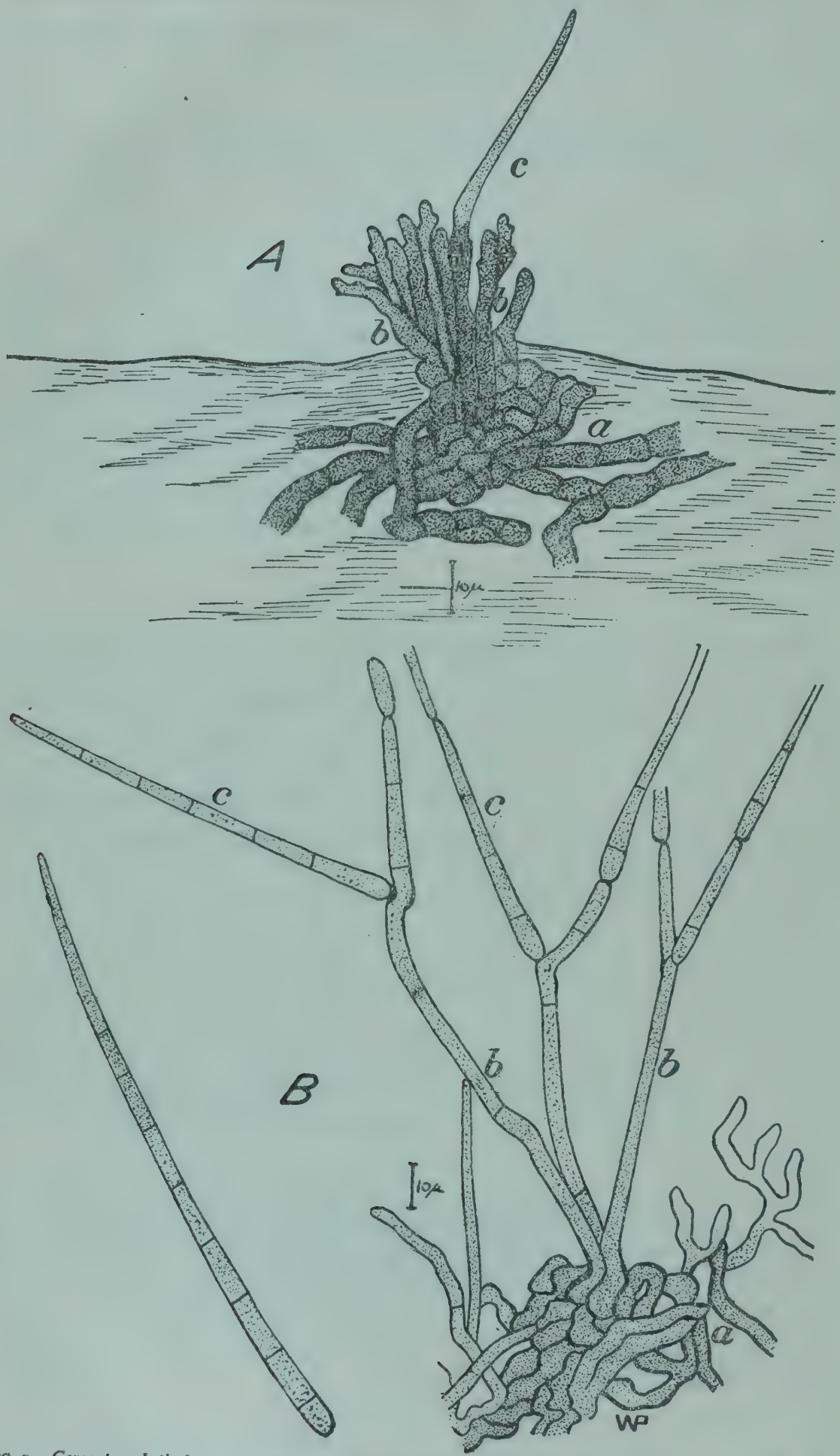


FIG. 1.—*Cercospora beticola: A*, Section of overwintered sugar-beet leaf showing embedded sclerotia-like body, *a*, with a mass of old conidiophores, *b*, from which a new conidium, *c*, was produced. *B*, Production of rather typical conidiophores, *b*, and conidia, *c*, from a sclerotia-like mass, *a*, taken from overwintered host material and placed in hanging-drop cultures.

### CONIDIA

Thümen (1886, p. 50–54)[1] believed that the spores of *Cercospora beticola* are able to live for a certain length of time in the soil and retain their viability and produce new infection, and Pammel (1891, p. 238–243) and Massee (1906, p. 52–53) accord with this view. In the investigations here considered it was found that when kept dry, as in the case of herbarium material, the conidia remained viable for 8 months (Table I, tests 10 to 13), but soon after that no growth occurred. Only rarely were conidia found on the infected areas of the leaves which were exposed to outdoor weather conditions, and such conidia seemed to lose their vitality soon after harvest. No germination was found to take place under optimum conditions in the case of conidia which had been thus exposed from 1 to 4 months (tests 14 and 15). However, conidia occasionally found on spots that had been well protected, for instance in the interior of a pile of hayed beet tops, retained their viability for from 5 to less than 12 months (tests 16 and 17). Since the conidia are rarely found after a short time even on infected material that has been well protected and since they rarely germinate after being exposed outdoors for even 1 month after harvest, it would seem that under ordinary field conditions they play no important part in the overwintering of the fungus.

TABLE I.—*Viability of the conidia of Cercospora beticola as affected by desiccation*

| Test No. | Environment. | Period of exposure. | Viability. |
|---|---|---|---|
| 1 | Stored, dry | 14 years | None. |
| 2 | .....do | 11 years | Do. |
| 3 | .....do | 10 years | Do. |
| 4 | .....do | 5 years | Do. |
| 5 | .....do | 4 years | Do. |
| 6 | .....do | 3 years | Do. |
| 7 | .....do | 2 years | Do. |
| 8 | .....do | 11 months | Do. |
| 9 | .....do | 10 months | Do. |
| 10 | .....do | 8 months | Slightly viable. |
| 11 | .....do | 7 months | Extremely viable. |
| 12 | .....do | 6 months | Do. |
| 13 | .....do | 5 months | Do. |
| 14 | Left in field after harvest, Colorado | 1 month | None. |
| 15 | Left in field after harvest, Wisconsin | 4 months | Do. |
| 16 | Stored inside pile of hayed sugar-beet leaves | 5 months | Extremely viable. |
| 17 | .....do | 12 months | None. |

### SCLEROTIA AND MYCELIUM

Various investigators have attempted to determine whether different fungi live in the soil over winter and the manner in which they overwinter. Treboux (1914) found that the mycelia of several different rusts overwinter on host material freely exposed to climatic conditions. Stewart (1913) placed in boxes of soil potato leaves and tubers infected with *Phytophthora infestans*, exposed them to outdoor winter conditions, and found that plants grown on such soil developed no blight. However,

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 60.

temperature and moisture conditions in boxes of soil exposed aboveground to winter conditions are much more varied than in soil at different depths in the field where normal overwintering usually occurs.

In the overwintering experiments here described the host material was kept in an environment comparable to ordinary field conditions. The experiments at Rocky Ford, Colo., were started about the middle of October, 1912, and continued for 11 months. In these experiments some of the infected material was mixed with soil, placed in boxes, and exposed aboveground during the winter (Pl. III, 1); a second portion was buried from 1 to 8 inches in the ground (Pl. III, 2), wire netting being used above and below the infected material to insure ready location when examinations were made for cultural tests (Pool and McKay, 1915); a third portion of the infected tops was placed in a pile on top of the ground (Pl. III, 3). During the experiment records were kept of soil and air temperatures, the former being taken at a depth of 5 inches and the latter being obtained from the Weather Bureau station at Rocky Ford.[1]

The experiments carried on near Madison, Wis., were started the last of November, 1913, and continued through the winter. Infected sugar-beet tops were buried in the soil at depths of 5 and 8 inches, while seed-beet stalks were left under ordinary conditions in the field. In this experiment also records were kept of soil and air temperatures, the former being taken from March until June at a depth of 5 inches and the latter obtained from the Weather Bureau station at Madison.

The effect of desiccation on material kept under herbarium conditions was to kill probably all life of the fungus within 12 months, as already shown, but material kept under an environment having more or less moisture accompanied by the disintegrating action of various organisms was affected in an entirely different manner, as will be shown. All cultures from the infected material used in the two experiments above outlined were made from definite leaf-spots. Although the diseased tissue was the last to be completely disorganized and consequently could be found as long as any portion of the leaf remained, it became more and more difficult to obtain such tissue as time went on.

The fungus was unable to survive six months' outdoor exposure in boxes of soil (Table II, experiment 2), and this was also true of the fungus on leaves which had been freely exposed to outdoor conditions—for instance, on the outside of a hayed pile of sugar-beet tops (experiment 3), and on leaves buried 6, 7, and 8 inches in the ground (experiments 19 to 23). In cultures from infected mother-beet stalks and leaves that had been left in the field for a time and then plowed under or stored there was no growth, or only an indefinite growth, of the fungus after 7 months (experiments 8 to 10), while in infected material that had been protected in the interior of a pile of hayed beet tops (experiment 4) and in material

[1] All the records included in this paper from the Weather Bureau station at Rocky Ford, Colo., were kindly furnished by Mr. P. K. Blinn, the local observer.

that had been slightly covered or buried from 1 to 5 inches in the ground the life of the fungus was entirely extinct after 12 months (experiments 11 to 18). The death of the fungus in material plowed under is due in all probability to the rapid disorganization which results under favorable temperature and moisture conditions, such, for instance, as those which prevailed at Rocky Ford through the winter of 1912–13. During that period there was insufficient moisture to permit severe freezing, but there was a daily extreme variation of soil temperature, indicating that the air temperature produced the changes through the more or less dry soil. In the experiments at Madison there was only a partial disintegration of the buried beet tops six months after harvest, but other factors impaired the vitality of the fungus and its life appeared to be entirely extinct; consequently, notwithstanding the great differences in soil factors, comparable results as to the life of the fungus were obtained from the experiments at both places.

TABLE II.—*Effect of desiccation and overwintering on the viability of Cercospora beticola in infected sugar-beet tops under field conditions at Rocky Ford, Colo., and Madison, Wis.*

| Experiment No. | Environment of sugar-beet-top material. | Period of exposure. | Number of spots from which cultures were made. | Number of viable spots. | Condition of leaves. |
|---|---|---|---|---|---|
| [a] 1 | Dried, stored: | | | | |
| | Illinois, Iowa | 14 years | 10 | 0 | Good. |
| | Connecticut | 11 years | 10 | 0 | Do. |
| | New York | 10 years | 10 | 0 | Do. |
| | Wisconsin | 5 years | 10 | 0 | Do. |
| | Iowa | 4 years | 10 | 0 | Do. |
| | Maryland | 3 years | 10 | 0 | Do. |
| | Colorado | 2 years | 10 | 0 | Do. |
| | New Jersey | 10 months | 10 | 3 | Do. |
| | Colorado | 9 to 11 months. | 20 | 10 | Do. |
| 2 | Stored in soil in boxes and left free under outdoor conditions, Colorado. | 2 months | 15 | 15 | Do. |
| | | 3 months | 7 | 7 | Do. |
| | | 4 months | 13 | 4 | Do. |
| | | 5 months | 12 | 2 | Do. |
| | | 5½ months | 12 | 0 | Do. |
| | | 7 months | 6 | 0 | Do. |
| 3 | From the outside of "hayed" pile of sugar-beet tops, Colorado. | 7 months | 6 | 0 | Do. |
| | | 10 months. | 13 | 0 | Do. |
| 4 | From the interior of "hayed" pile of sugar-beet tops, Colorado. | 2 months | 10 | 10 | Do. |
| | | 3 months | 66 | 64 | Do. |
| | | 4 months | 29 | 27 | Do. |
| | | 5 months | 40 | 40 | Do. |
| | | 7 months | 18 | 12 | Do. |
| | | 10 months. | 15 | 10 | Do. |
| | | 12 months. | 25 | 0 | Do. |
| 5 | In field, Colorado | 2 months | 10 | 10 | Do. |
| | | 5 months | 11 | 8 | Do. |
| | | 8 months | 10 | 2 | Do. |
| 6 | Leaves from "mother beet" stalks free in field, Wisconsin. | 5 months | 21 | 15 | Do. |
| 7 | First-year sugar-beet leaves free in field, Wisconsin. | 5 months | 32 | 3 | Do. |
| | | 8 months | 40 | 0 | Partially disintegrated. |
| 8 | Spots on "mother beet" stalks free in field, Wisconsin. | 4 months | 7 | [b] 7 | Good. |
| | | 7 months | 35 | 4? | Do. |
| 9 | Spots on "mother beet" stalks free in field 6 months, then plowed under 1 month, Wisconsin. | 7 months | 10 | 0 | Somewhat softened. |

[a] Herbarium specimens for this test were furnished by Barrett, Illinois; Clinton, Connecticut; Whetzel, New York; Pammel, Iowa; Norton, Maryland; and Cook, New Jersey.

[b] 395 colonies.

TABLE II.—*Effect of desiccation and overwintering on the viability of Cercospora beticola in infected sugar-beet tops under field conditions at Rocky Ford, Colo., and Madison, Wis.*—Continued

| Experiment No. | Environment of beet-top material. | Period of exposure. | Number of spots from which cultures were made. | Number of viable spots. | Condition of leaves. |
|---|---|---|---|---|---|
| 10 | Spots on "mother beet" stalks free in field 4 months, then stored dry 3 months, Wisconsin. | 7 months.. | 10 | 5? | Good. |
| 11 | Buried 1 inch in ground, Colorado.... | 6 months.. | 21 | 0 | Partially disintegrated. |
| | | 7 months.. | 10 | 2 | Do. |
| | | 10 months. | 8 | 3 | Greatly disintegrated. |
| | | 12 months. | 24 | 0 | Entirely disintegrated. |
| 12 | Buried 2 inches in ground, Colorado.. | 5 months.. | 14 | 2 | Partially disintegrated. |
| | | 6 months.. | 19 | 10 | Do. |
| | | 10 months. | 11 | 0 | Greatly disintegrated. |
| | | 12 months. | 28 | 0 | Entirely disintegrated. |
| 13 | Buried 3 inches in ground, Colorado.. | 6 months.. | 21 | 10 | Partially disintegrated. |
| | | 10 months. | 11 | 1 | Greatly disintegrated. |
| | | 12 months. | 18 | 0 | Entirely disintegrated. |
| 14 | Broken and buried 3 inches in ground, Colorado. | 6 months.. | 14 | 0 | Greatly disintegrated. |
| | | 7 months.. | 12 | 5 | Do. |
| | | 10 months. | 16 | 0 | Entirely disintegrated. |
| 15 | Buried 4 inches in ground, Colorado.. | 6 months.. | 19 | 5 | Partially disintegrated. |
| | | 10 months. | 19 | 1 | Greatly disintegrated. |
| 16 | Buried 5 inches in ground, Colorado.. | 6 months.. | 20 | 4 | Do. |
| | | 10 months. | 15 | 0 | Entirely disintegrated. |
| | | 12 months. | 20 | 0 | Do. |
| 17 | Buried 5 inches in ground, Wisconsin. | 4 months.. | 30 | 0 | Good. |
| | | 5 months.. | 30 | 3? | Do. |
| | | 6 months.. | 81 | 0 | Do. |
| | | 7 months.. | 80 | 0 | Partially disintegrated. |
| 18 | Plowed under in field, about 5 inches, Wisconsin. | 5 months.. | 30 | 3? | Do. |
| | | 5 months.. | 20 | 0 | Do. |
| 19 | Buried 6 inches in ground, Colorado.. | 5 months.. | 8 | 0 | Greatly disintegrated. |
| | | 6 months.. | 20 | 4 | Do. |
| | | 10 months. | 12 | 0 | Do. |
| | | 12 months. | 15 | 0 | Do. |
| 20 | Broken and buried 6 inches in ground, Colorado. | 6 months.. | 18 | 0 | Do. |
| | | 7 months.. | 10 | 0 | Do. |
| | | 10 months. | 16 | 0 | Entirely disintegrated. |
| 21 | Buried 7 inches in ground, Colorado.. | 6 months.. | 15 | 0 | Do. |
| | | 7 months.. | 10 | 0 | Do. |
| | | 10 months. | 12 | 0 | Do |
| | | 12 months. | 22 | 0 | Do |
| 22 | Buried 8 inches in ground, Colorado.. | 6 months.. | 20 | 0 | Do |
| | | 7 months.. | 10 | 0 | Do. |
| | | 10 months. | 12 | 0 | Do. |
| 23 | Buried 8 inches in ground, Wisconsin. | 4 months.. | 30 | 0 | Good; ground frozen. |
| | | 5 months.. | 35 | 2? | Good. |
| | | 6 months.. | 57 | 0 | Good; leaves have sour odor. |
| | | 6½ months | 100 | 0 | Partially disintegrated. |

These experiments and observations made in the field during several spring and summer months showed that on leaves slightly protected on or near the surface of the ground during the winter *C. beticola* can live a sufficient length of time to be a source of infection for the succeeding sugar-beet crop and that the fungus is entirely killed by planting time when the infected material is plowed under to a depth of 6 to 8 inches in the fall.

## AIR AND SOIL TEMPERATURES AT ROCKY FORD, COLO., AND AT MADISON, WIS.

Comparison of the air and soil temperatures which prevailed during the experiment at Rocky Ford and Madison showed a wide difference.

One of the most striking characteristics of these temperatures at Rocky Ford was the wide range between the maximum and the minimum, and this range may be observed throughout the entire records (fig. 2, 3). In the case of the soil temperatures especially, the wide range appeared to be due to a lack of moisture, the extreme variations being greater than if more moisture had been present. A comparison of the records shows that the variation in air temperature was much less and the mean daily temperature constantly lower at Madison than at Rocky Ford, notwithstanding the fact that the daily minimum temperature was usually lower at Rocky Ford. A comparison of the soil temperatures at the two points, however, shows that at Madison it probably remained more constant and was never as low as at Rocky Ford. This was due apparently to the greater amount of moisture in the soil at Madison and consequently its continued frozen condition. After March 23, the date on which the record was begun at Madison, the soil temperature at that place was never below 29° F., notwithstanding the fact that the air temperature was as low as 15° on April 8, while the minimum soil temperature at Rocky Ford was 22° on December 21 and 25° on February 8. However, as the air temperature on these dates was lower here than at Madison, comparisons can not be drawn too closely.

In view of the presence of snow on the ground, which, as is well known, protects the soil from the extreme variations of air temperature, and the prevailing low air temperatures, as shown by the records, it may be assumed that the soil temperatures at Madison during January and February and the early part of March varied but little from freezing. This assumption is supported by Frödin's experiments (1913), which showed in general that when the air temperature was much lower than that of the soil the soil temperature in ground covered with snow was higher than in bare ground. He found that temperatures taken at a depth of 10 cm. in the former were the same as those taken at a depth of 27.4 cm. in the latter. After the early part of April the minimum soil temperatures at Rocky Ford and Madison agreed closely, although the minimum air temperature at the former place remained generally the lowest of the temperatures recorded.

Temperatures obtained from the interior of a pile of hayed sugar-beet leaves by means of a soil thermograph buried in the pile varied less than temperatures taken outside the pile, as shown by the following records made on May 8, 1913, and as was probably the case during the entire winter season: Temperature inside pile, maximum, 67° F.; minimum, 58°; difference, 9°. Temperature outside pile, maximum, 84° F.; minimum, 45°; difference, 39°.

In view of the fact that the fungus lived twice as long inside the pile as it did on the outside it would seem that a more uniform temperature might be regarded as one of the controlling factors in the life of the fungus.

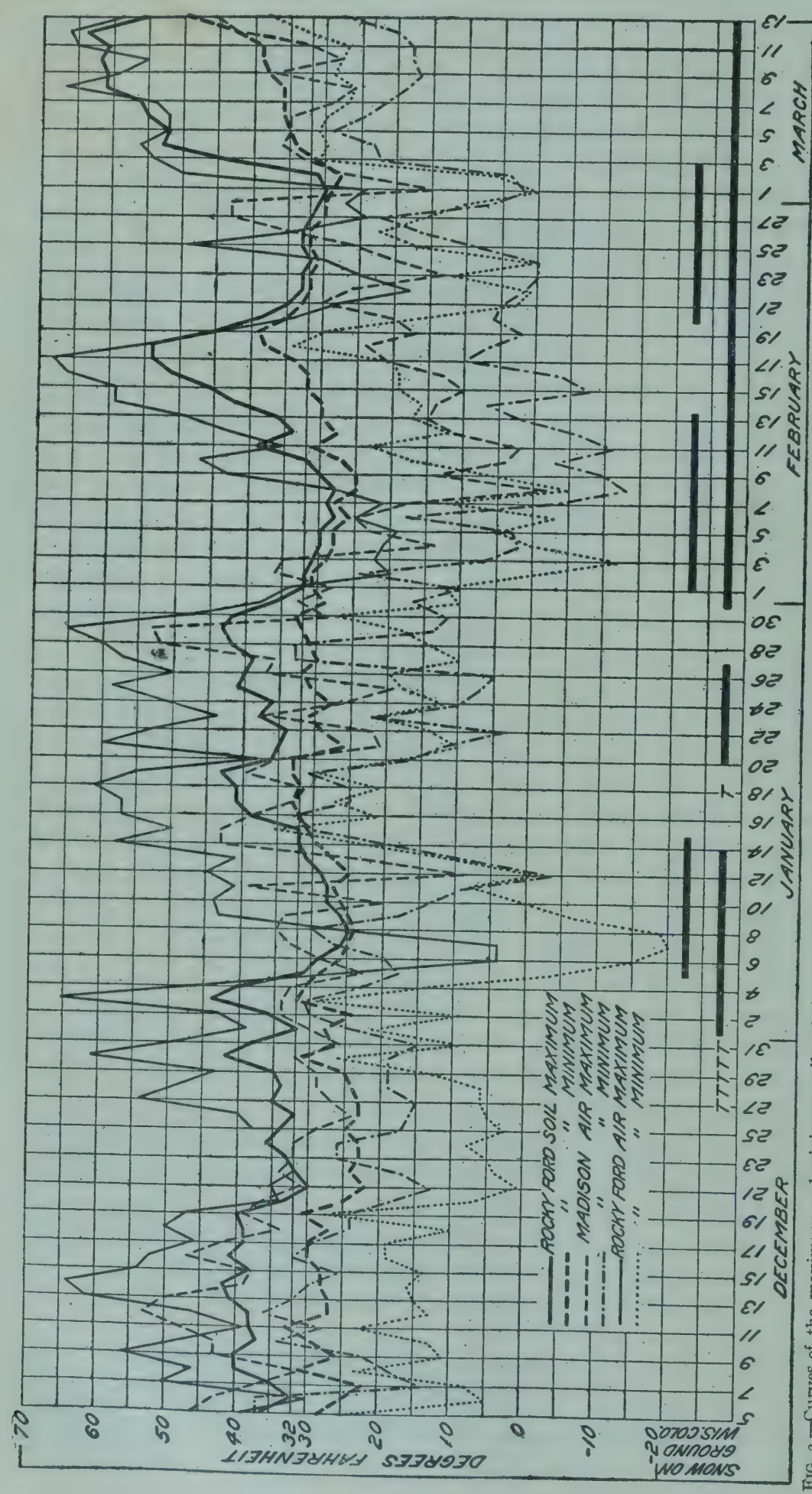


FIG. 2.—Curves of the maximum and minimum soil and air temperatures for the period from December 5, 1912, to March 13, 1913, at Rocky Ford, Colo., and air temperatures from December 5, 1913, to March 13, 1914, at Madison, Wis., together with the periods that snow covered the ground.

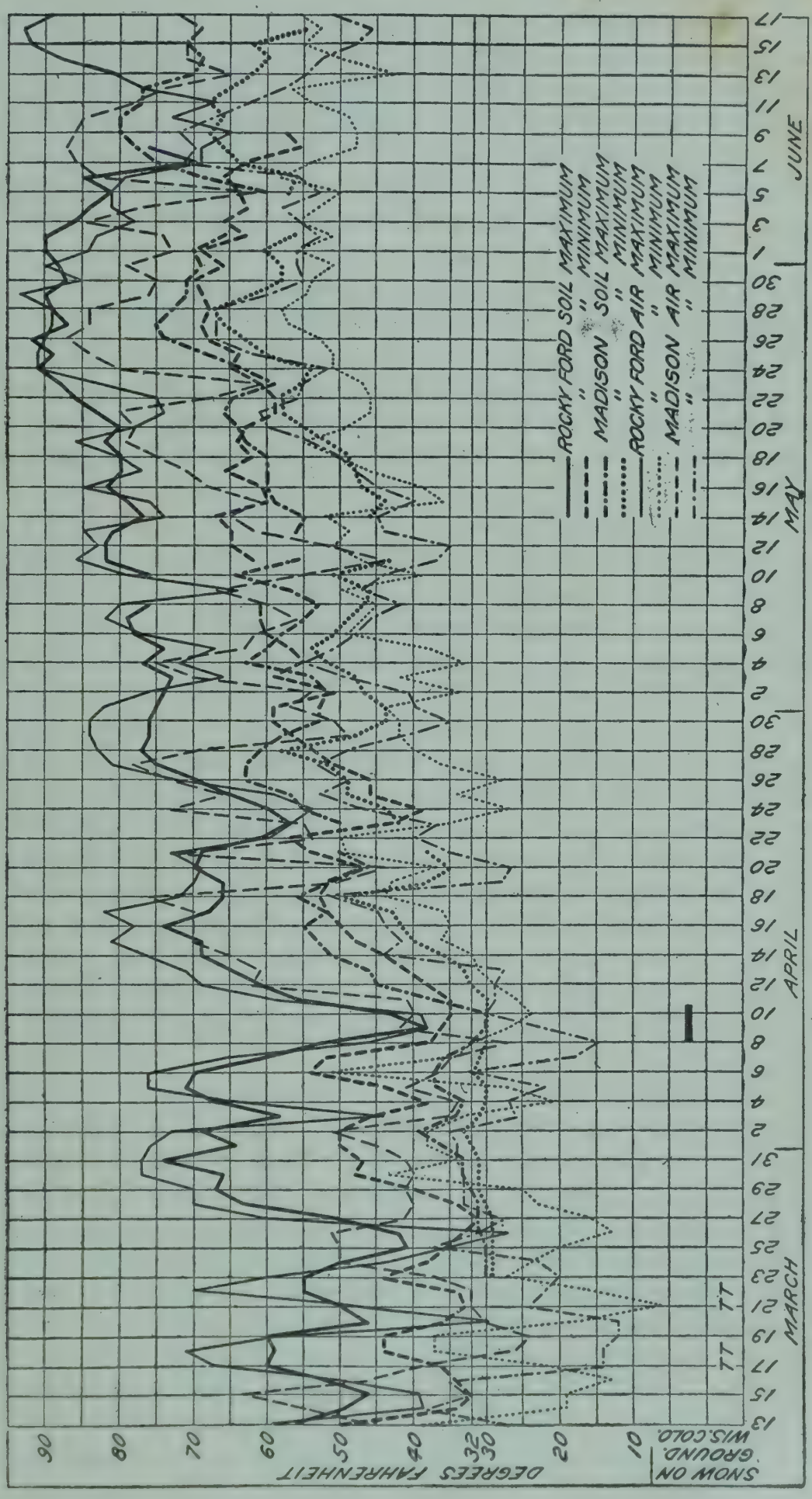


FIG. 3.—Curves of the maximum and minimum soil and air temperatures for Rocky Ford, Colo., from March 13 to June 17, 1913, and for Madison, Wis., from March 13 to June 17 1914.

Low temperatures are not entirely inhibitive, as was shown by thermal tests of artificial cultures. After such cultures had been exposed to temperatures averaging 0.9° C. for 48 days and then kept at 28° C., numerous colonies developed. Also, heavily infected leaves kept at 0.9° C. for 97 days yielded good growth when cultures were made and held at favorable temperatures. Had the cultures been exposed to freezing temperatures or to extreme variations in temperature, the effect would doubtless have been more pronounced.

Although the temperature variations and the amount of soil moisture at Rocky Ford and Madison differed greatly, the effect on the life of the fungus was apparently the same at both places. It may be concluded that conditions of the soil which favor the process of disintegration are the most important factors in the control of the disease, and these experiments indicate that these processes are most active at a depth of 6 to 8 inches.

## SUMMER CLIMATIC CONDITIONS

The summer climatic conditions here considered were recorded during 1913 in fields of first-year sugar beets grown at Rocky Ford, these fields being an example of the usual progress of the disease where neither rotation nor sanitation at the preceding harvest time had been practiced.

A study of the temperature and humidity records taken at different places in a beet field at Rocky Ford and at the Weather Bureau station 3 miles from the field was made to determine their comparative values in making important correlations. The records made in the sugar-beet field were taken by means of hydrothermographs kept in meteorological instrument shelters 5 feet above the ground (Pl. IV, fig. 1) and among the plants (Pl. IV, fig. 2). These were checked at frequent intervals with a sling and cog psychrometer (Shaw, 1914), respectively, and under Colorado conditions were found to be accurate. The records of the Weather Bureau station were taken by means of maximum and minimum thermometers kept in an instrument case about 5 feet above the ground in an open space (fig. 3).

The daily maximum and minimum temperatures and humidities, together with the total number of hours the humidity was above 60 from noon of the preceding day to noon of the given day, are used in the present interpretations. It has been found that when a high relative humidity prevails, the stomata of the sugar-beet leaves are usually open; and as the fungus enters the leaves only through the open stomata, the length of time they remain open is a fundamental factor in determining the possible occurrence of infection (Pool and McKay, 1916).

### AIR TEMPERATURE AND RELATIVE HUMIDITY

The temperature and relative humidity taken with hygrothermographs placed near the ground among the plants varied widely from those taken with hygrothermographs in the air above the field and also from those

taken at the Weather Bureau station; hence, the place where the records were taken for use in the present correlations with the development of the disease is an important consideration.

AIR TEMPERATURE.—At Rocky Ford the maximum temperatures taken among the plants near the surface of the ground from June 13 to 30 ranged from 2 to 19 degrees higher and the minimum temperatures generally from 1 to 14 degrees lower than those taken at 5 feet above the ground (fig. 4). This was due to the fact that the plants were small during this period and covered only a portion of the ground; consequently during the daytime the temperature of the soil became higher than that of the air, and in turn the temperature of the air near the ground became higher than that of the air a few feet above. During the night the reverse occurred, the surface soil losing its heat by radiation and conduction faster and finally reaching a lower temperature than that of the air in contact with it, after which the heat of the latter gradually passed into the soil and as a result the temperature of the air immediately above the ground eventually became lower than that a few feet higher up. It is possible that convection currents also tended to lower the temperature of the air immediately above the ground; for, as is well known, when it is not disturbed by other factors, the coolest air settles to the lowest levels.

The maximum temperatures of the air near the ground, as shown by the records, were higher for a longer period during June than at any time during the season, varying from 100° to 106° F. on nine different days between the 14th and 26th of that month and rising above 100° only once thereafter, on August 16. The maximum temperature of the air 5 feet above the ground, on the other hand, was lower during June than during the middle of the season, ranging from 90° to 93° on six different days during the month, while it was above 90° and sometimes as high as 100° on 12 different days during July.

As shown by the records, the temperature of the air near the ground among the plants was lower during the middle than during the early part of the season. This was probably due to the difference in the size of the plants, the larger plants practically covering the ground in midseason and preventing the heating of the surface soil, while early in the season the smaller plants covered the ground but sparsely and consequently afforded less protection against heating. Comparison of the records also shows that during the middle of the season the temperature of the air among the plants near the ground was practically the same as that of the air 5 feet above the field and that throughout the entire period the latter was quite comparable with the temperatures taken at the Weather Bureau station (fig. 4).

A similar marked variation was shown at Madison, the maximum temperature there being almost constantly higher and the minimum tempera-

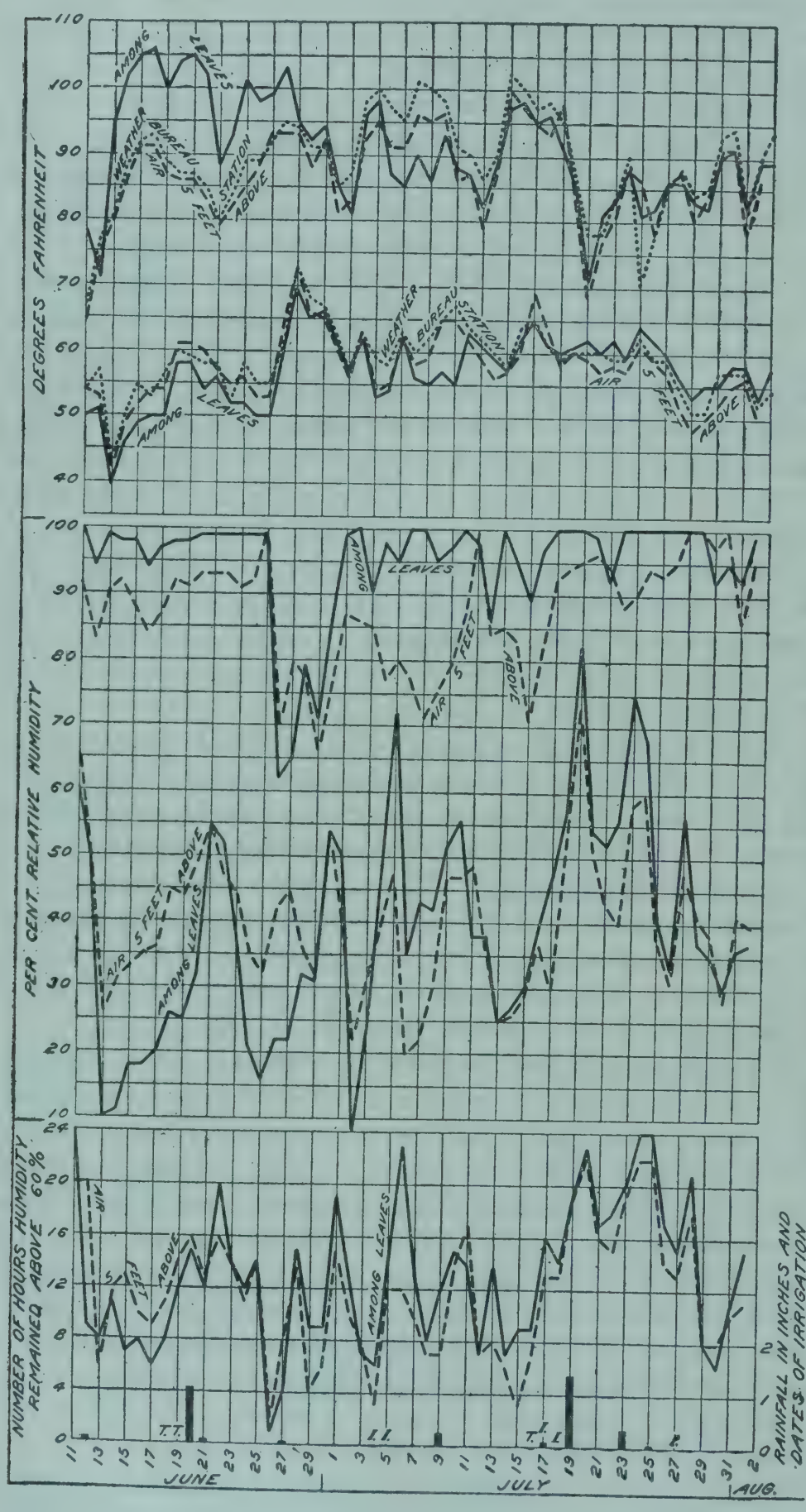


FIG. 4.—Curves of the maximum and minimum temperatures and relative humidities and the number of hours that the humidity remained above 60 from noon of the preceding to noon of the given day among sugar-beet plants and in the air 5 feet above the field, together with the field rainfall and irrigation records. For comparison, the maximum and minimum temperature records from the Weather Bureau station are included. June 11 to August 2, 1913, at Rocky Ford, Colo.

ture usually lower among the beet plants than the temperature shown by the Weather Bureau records, which were taken on top of a four-story building about a mile from the sugar-beet field. These wide variations between the air temperature taken near the ground among the plants and that taken 5 feet above the field and between the former and the temperature taken at the Weather Bureau stations show that for correlation with fungous activities only the records taken among the plants should be used.

RELATIVE HUMIDITY.—There was also a wide variation in the humidity near the ground among the plants and 5 feet above the field. For instance, the daily minimum humidity at Rocky Ford from June 13 to 29, with two exceptions, was higher and remained above 60 generally for a longer period in the air above the plants than among the leaves near the ground (fig. 4), owing to the higher temperature at the surface of the ground as a result of the small amount of covering afforded by the young plants. During this period the daily variation of humidity among the leaves was extreme, ranging from 99 to 10 on June 13, from 99 to 16 on June 25, and from 100 to 8 on July 2. After June 29, on the other hand, the minimum humidity was generally higher, the humidity remained above 60 for a longer time among the leaves than in the air above, and the daily variation among the leaves was less extreme than earlier in the season. These conditions were due mainly to the greater amount of covering afforded by the larger plants and consequent longer retention of moisture among the leaves. The humidity both among the plants and in the air 5 feet above the field remained, on an average, above 60 for a longer time each day during midsummer than during June, owing in part to the increased use of irrigation water as the season advanced and the increased amount of moisture in the surrounding air resulting from the increased transpiration of the larger plants.

Comparison of the Madison and the Rocky Ford records (fig. 5) of the number of hours that the relative humidity remained above 60 each day among the sugar-beet plants shows that throughout the season it was higher, on an average, at Madison. Here it remained above 60 for a longer time each day during the latter half of June, when the records were started, and for a shorter time each day during August than during any other summer month. This was due to difference in the amount of rainfall, there being frequent rains during the former period and comparatively dry weather during the latter. At Rocky Ford the facts were reversed, the humidity remaining above 60 for a longer time each day during midseason than during the latter half of June or the first part of September. This was probably due to more frequent irrigation and the increased covering afforded by the larger plants of midseason.

Table III shows the average number of hours a day that the relative humidity remained above 60 at Madison and at Rocky Ford.

TABLE III.—*Average number of hours a day that the relative humidity was above 60 at Madison, Wis., and Rocky Ford, Colo., during the summer of 1914 and 1913, respectively*

| Date. | Madison, Wis. (1914). | Rocky Ford, Colo. (1913). |
|---|---|---|
| | *Hours.* | *Hours.* |
| June 16 to 30 | 19.4 | 10.8 |
| July | 17.3 | 14.2 |
| August | 16.6 | 14.1 |
| September 1 to 6 | 18.0 | 11.3 |
| Seasonal average | 17.4 | 13.4 |

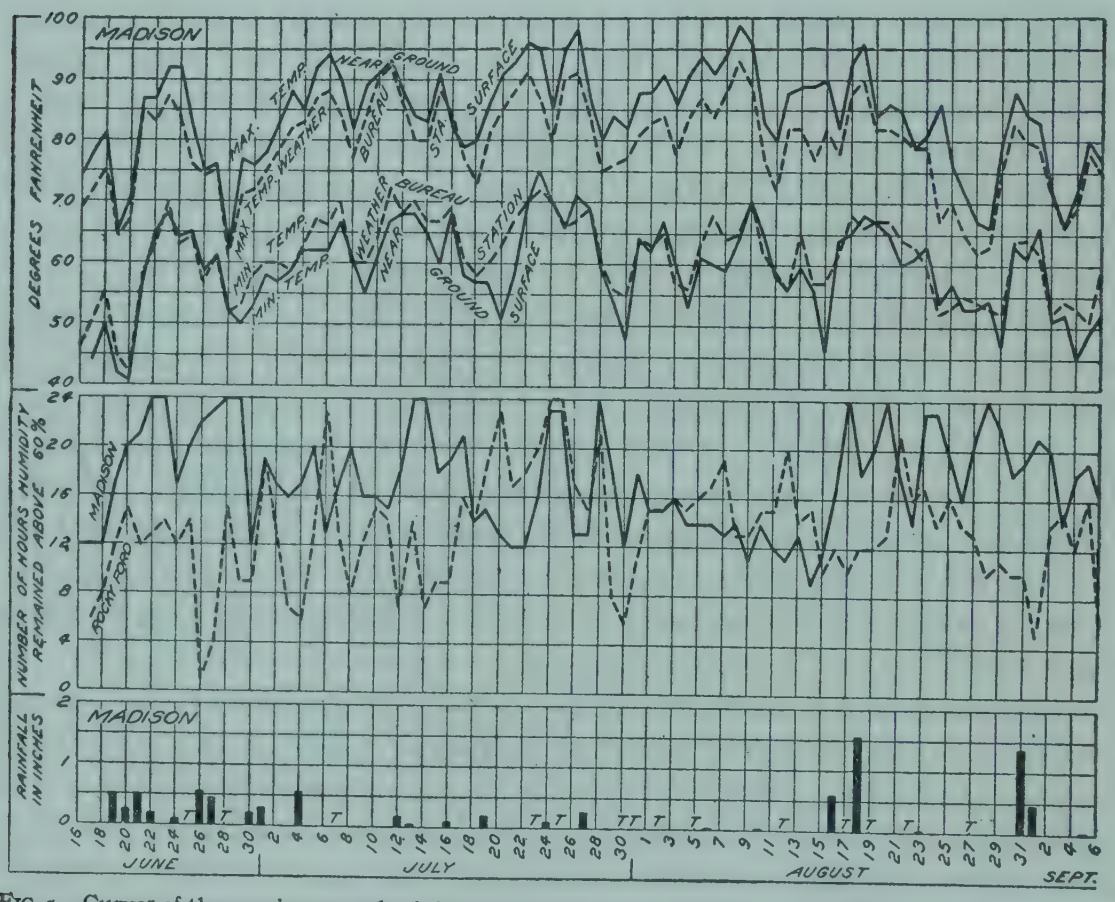


FIG. 5.—Curves of the maximum and minimum temperatures among sugar-beet plants and at the Weather Bureau station, and the seasonal rainfall records at Madison, Wis., in 1914, and the number of hours that the humidity remained above 60 among the sugar-beet plants in the field at Madison, Wis., in 1914, and at Rocky Ford, Colo., in 1913.

The greater average number of hours of high humidity at Madison accounts for the periods of extreme infection which occurred there when the fungus was present. Here leaves badly infected with *Cercospora beticola* and entirely covered with conidia were found at times, but this condition was rarely seen at Rocky Ford. There were numerous cases of cotyledon infections also at Madison, the high humidity early in the season favoring their occurrence; but no such infections were found at Rocky Ford.

## RAINFALL AND IRRIGATION

The rainfall records made during the summer season of 1913 in the beet field at Rocky Ford in which infection was studied in detail (fig. 4, 7) where obtained by means of a rain gauge placed at the edge of the sugar-beet field (Pl. IV, fig. 1). Most of the rain was in the form of local showers, the amount varying greatly within a radius of less than 2 miles; but occasionally general rains fell. The effect of the increased relative humidity resulting from rainfall usually lasted longer among the leaves than in the air 5 feet above (fig. 7).

The effect of irrigation on humidity was found to be similar to the effect of rain. On July 2, before the field was irrigated, its humidity was as low as 8 and on July 3 and 4 remained above 60 for 7 and 6 hours, respectively. On July 4 and 5 the field was irrigated and the humidity remained above 72 on the 4th and above 60 during 23 hours of the 5th. On July 27 the field was again irrigated and the humidity remained above 60 for 15 hours that day and 21 hours the following day. On August 19 and 20 the field was irrigated the third time and the humidity remained above 60 for 12 and 13 hours, respectively, and the next day 21 hours. The general humid conditions necessary for leafspot infection, however, are developed much better by rain than by irrigation, because of the latter being comparatively local and unaccompanied by the atmospheric conditions attending rainfall.

## WIND

Records of wind velocity at Rocky Ford were taken by means of an anemometer placed 6 feet in the air at the edge of the beet field (Pl. IV, fig. 1), the readings being made at irregular intervals and the velocities computed being the hourly averages from one reading to the next. As the records were not made daily, accurate hourly velocities for different intervals during the day can not be obtained from the records. They show, however, that the average seasonal velocity from June 12 to September 22 was 5.3 miles per hour. Occasionally two daily readings were made, one in the morning and the second late in the afternoon. These show that the average velocity of the wind was always higher during the day than at night, the greatest velocity usually prevailing in the afternoon during the period of lowest humidity. While no general dissemination of conidia was correlated with high wind velocity, the afternoon combination of highest wind with lowest humidity apparently favored the dissemination of conidia. In fact, in the case of air cultures made at different times during several days, it was found that the fungus grew usually only on those exposed during the afternoon.

## SUMMER INFECTION CYCLES

The thermal relations of the fungus are closely linked with the effect of various climatic factors on the production and dissemination of conidia and on infection cycles. With a view to determining these relations the fungus was grown in Petri-dish cultures in thermostats at different and varied temperatures. At first the moisture was probably more or less constant, but as time went on it became relatively low. The effect of different temperatures, however, was comparable with that observed under existing field conditions.

### THERMAL RELATIONS OF THE FUNGUS IN CULTURES

Tests of the fungus on string-bean agar were made at Washington during November and December, 1913, and January, 1914. The cultures were obtained from isolations made at the time of the tests from infected sugar-beet leaves collected at Rocky Ford during the preceding September. One colony of the first isolations was macerated in 10 c. c. of sterile water, and one platinum loop of this suspension was used for each tube of medium. Three poured plates were used for each single test. The cultures were exposed to different constant temperatures and to varied constant temperatures (high and low changed to low and high, respectively). Exposures were also made for 8 hours at the higher temperatures and then for 16 hours at lower temperatures, and, after a short interval of exposure in a certain number of these tests, both temperatures were lowered, it being possible in this way to approximate night and day temperatures in the field under normal conditions.

SERIES A (DIFFERENT CONSTANT TEMPERATURES).—When the cultures were held at different constant temperatures, the abundance and size of the individual colonies gradually increased, while the time necessary for development decreased with the temperatures 12.5°, 17.3°, 19.2°, 20°, and 30.8° C. The best growth was made at a temperature of 30.8°, but this in all probability was slightly above the optimum constant temperature, as no growth took place in cultures held for 9 days at 34.7°, 35.8°, and 40.6°, respectively (Table III, series A).

SERIES B (VARIED CONSTANT TEMPERATURES).—Although no growth of the fungus took place in cultures held at constant temperatures of 34.7° and 35.5° C., a small percentage of normal colonies developed in cultures exposed for three days to these temperatures and then for several days to a temperature of 30.8°, while in cultures exposed for three days to 40.5° no growth occurred when subsequently held at 30.8°. On the other hand, in cultures exposed for three days to a temperature of 30.8° there was almost a normal development of the colonies for three days after they were exposed to 34.7°, but at the end of five days the inhibitive effect of the latter temperature became manifest. In the case of cultures

changed from 30.8° to 40.5° only a very slight increase in growth was apparent during the first three days at the higher temperature, and after that it ceased entirely (Table III, series B).

TABLE III.—*Comparative diameter of colony growth (in millimeters) of Cercospora beticola at different constant temperatures,[a] at decreasing and increasing constant temperatures, and at daily varied temperatures*

SERIES A, CONSTANT TEMPERATURES

| | Diameter of colony growth at temperature (°C.) of— | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Maximum | 2.1 | 7.9 | 11.1 | 14.4 | 19.1 | 20.2 | 21.0 | 33.0 | 36.2 | 37.4 | 41.0 |
| Minimum | 0.3 | 4.0 | 8.0 | 11.5 | 16.3 | 18.6 | 19.5 | 29.7 | 33.8 | 34.5 | 40.0 |
| Average | 0.9 | 5.4 | 8.9 | 12.5 | 17.3 | 19.2 | 20.0 | 30.8 | 34.7 | 35.8 | 40.6 |
| Period of growth: | | | | | | | | | | | |
| 3 days | 0 | 0 | 0 | 0 | 0.25 | 0.65 | 0.67 | 3.6 | 0 | 0 | 0 |
| 6 days | 0 | 0 | 0 | .25 | .8 | 2.2 | 2.3 | 6.4 | 0 | 0 | 0 |
| 9 days | 0 | 0 | .25 | .6 | 1.5 | 5 | 4.6 | 7 | [b] 0 | [b] 0 | [b] 0 |
| 11 days | 0 | 0 | .4 | .85 | 2.8 | 7 | 6.4 | ..... | ..... | ..... | ..... |
| 14 days | 0 | 0 | .5 | 1.5 | 4.2 | ..... | ..... | ..... | ..... | ..... | ..... |
| 18 days | 0 | 0 | .7 | 2.2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 22 days | 0 | 0 | 1.2 | 3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

[a] The temperatures of each thermostat for all tests were averaged from two daily readings continued throughout the time of the experiment.
[b] No growth occurred in these plates when held at 28° C. for 10 days.

SERIES B, DECREASING AND INCREASING CONSTANT TEMPERATURES.

| | Diameter of colony growth at temperature (°C.) of— | | | | |
|---|---|---|---|---|---|
| | [a] 34.7 | [a] 35.5 | [a] 40.5 | [b] 30.8 | [c] 30.8 |
| Period of growth: | | | | | |
| 3 days | 0 | 0 | 0 | 3.6 | 3.4 |
| 6 days | 4 | 3.6 | 0 | 6 | 3.7 |
| 8 days | [d] 8.3 | [e] 9.3 | 0 | 6.4 | 3.7 |

[a] Temperature changed to 30.8° C. after three days.
[b] Temperature changed to 34.7° C. after three days.
[c] Temperature changed to 40.5° C. after three days.
[d] Only 19.2 per cent of the normal number of colonies developed.
[e] Only 12.8 per cent of the normal number of colonies developed.

SERIES C, DAILY VARIED TEMPERATURES

| | Diameter of colony growth at temperature (°C.) of— | | | | | | |
|---|---|---|---|---|---|---|---|
| 16 hours at | 14.5 | 14.5 | 14.5 | 20 | 20 | 20 | 20 |
| 8 hours at | 19.2 | 21.6 | 28 | 30.8 | 34.7 | 35.8 | 40.6 |
| Period of growth: | | | | | | | |
| 3 days | 0.4 | 0.5 | 0.7 | 1.7 | 0.6 | 0.6 | 0 |
| 5 days | .7 | 1.9 | 3.4 | 6 | 3.6 | 3.2 | 0 |
| 7 days | 2.7 | 3.6 | 5.2 | 8.8 | 4.8 | 5.4 | 0 |
| 9 days | ..... | ..... | ..... | ..... | ..... | ..... | 0 |

SERIES C (DAILY VARIED TEMPERATURES).—In these tests the temperatures were made to correspond closely with summer outdoor temperatures of night and day by holding the cultures for 16 hours at the lower and for 8 hours at the higher. After seven days' exposure the growth of colonies

on cultures exposed to temperatures of 14.5° and 19.2° C. averaged 2.7 mm. in diameter; after exposure for the same length of time to 14.5° and 21.6°, 14.5° and 28°, and 14.5° and 30.8° the growth gradually increased until it reached a maximum diameter of 8.8 mm; but when exposed to higher temperatures (20° and 34.7° or 20° and 35.8°) the growth gradually diminished until finally it equaled approximately that attained under 14.5° and 28°. There was no growth on cultures exposed for nine days or longer to 20° and 40.6° (Table III, series C).

SERIES D (HIGH VARIED CHANGED TO LOW VARIED TEMPERATURES).—A plate culture exposed for three days to temperatures of 20° and 40.5° C., being held 16 hours at the lower and 8 hours at the higher, and then for six days at 20° and 30.6°, developed 23 colonies, averaging 10.3 mm. by the end of the latter period, while a check plate exposed constantly to a temperature of 30.6° developed 100 colonies by the end of the latter period. A plate exposed to the higher temperatures—20° and 40.5°—for five days and then held at 20° and 30.6° for six days developed six colonies at the end of the latter period, while a plate exposed to 20° and 40.5° and then held at 20° and 30.6° for seven days developed no growth of the fungus.

Later on in this paper the fact that high minimum and maximum temperatures inhibit the growth of the fungus, as brought out by these tests, is correlated with the effect of existing high field temperatures, with their consequent accompanying factors, on the leaf spot. Although the optimum temperature variations—20° and 30.8° C.—were found to be very favorable to the development of leafspot in the field, little or no increase in the disease was observed to follow high night and day field temperatures—20° and 40.5°, respectively.

It was also observed that different temperatures affect conidial septation. The normal average septation varies from 6 to 11, but during warm, humid periods the conidia were usually found to be many septate, sometimes as high as 20-septate, while after a cooler period, such as usually occurs in September, they were only from 2- to 4-septate.

## RELATION OF CONIDIAL PRODUCTION AND DISSEMINATION TO CLIMATIC CONDITIONS

For the purpose of studying the relation of temperature and relative humidity to the production and dissemination of conidia, detailed life histories of a large number of individual spots on 10 plants in the medium-early field at Rocky Ford were kept during the season of 1913. The temperature and humidity records used in these correlations were those taken among the beet leaves near the ground and, together with rainfall and dates of irrigation, are shown in figure 7.

Beginning with the outermost or oldest, the leaves were tagged and numbered consecutively, and the location of the spots on each was indi-

cated on diagrams. As new leaves developed, they were included in the observations, and this was true also of new spots, until they became too numerous, after which only a few representative ones on each leaf were studied in detail. During the period from the 24th of June to the 19th of September 330 spots were studied, both surfaces being examined at frequent intervals with a hand lens. For the purpose of getting a basis for comparison of rates of development at different stages in the life history of the disease, percentage values were assigned to each stage as follows, the spots being grouped and averaged later (Table IV):

| Percentage value. | Stage of development of fungus. |
|---|---|
| 5.0 | Spot first noticed. Neither conidia nor conidiophores present. |
| 12.5 | Conidiophores present. |
| 19.7 | Very few conidia. |
| 25 | Few conidia. |
| 31.2 | Conidia fairly numerous. |
| 37.5 | Conidia numerous. |
| 43.7 | Conidia fairly abundant. |
| 50 | Conidia abundant. |

The value of the spot is the sum of the values of the two sides—that is the value of a spot on which there were but few conidia (25) on one side, and abundant conidia (50) on the other, is 75. Again, the value of a spot on which conidiophores only (12.5) were present on one side, and very few conidia (19.7) on the other, is 32.[a]

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*[b]

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| **2 | 3 | 5 | July 7 | 10 | No conidiophores on either surface. July 8, no change. |
| | | | 10 | 37 | Conidiophores above, conidia few below. |
| | | | 12 | 50 | Conidia few on both surfaces. July 14, no change. |
| | | | 16 | 50 | Conidia very few above, fairly numerous below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| | | | 25 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 28 | 37 | Conidia none above and few below. |
| 2 | 4 | 1 | 2 | 10 | No conidiophores on either surface. July 7, no change. |
| | | | 8 | 24 | Conidiophores on both surfaces. |
| | | | 10 | 69 | Conidia very few above and abundant below. |
| | | | 12 | 75 | Conidia few above and abundant below. |
| | | | 14 | 100 | Conidia abundant on both surfaces. July 16, 21, 23 (leaf yellow), July 25, no change. |
| | | | 28 | 63 | Conidia numerous above and few below. |
| 2 | 4 | 3 | 7 | 10 | No conidiophores on either surface. July 8, no change. |
| | | | 10 | 25 | Conidiophores on both surfaces. |
| | | | 12 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 14 | 82 | Conidia numerous above and fairly abundant below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. |
| 2 | 4 | 4 | 7 | 10 | No conidiophores on either surface. |
| | | | 8 | 25 | Conidiophores on both surfaces. |
| | | | 10 | 75 | Conidia few above and very abundant below. July 14, no change. |
| | | | 16 | 57 | Conidia very few above, numerous below. |

[a] For convenience the decimal fractions, which make only a negligible difference in the averages, are omitted.

[b] In Table IV asterisks (*), daggers (†), and section marks (§) are used to designate definite leaf spots to which reference is made in the text.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| **2 | 6 | 1 | July 7 | 15 | Conidiophores forming above, nothing below. |
| | | | 8 | 31 | Conidiophores above, conidia very few below. |
| | | | 10 | 100 | Heavy production of conidia on both surfaces. July 12, 14, 16, 21, 23, no change. |
| | | | 25 | 63 | Conidia few above and numerous below. |
| 3 | 2 | 1 | June 24 | 10 | No conidiophores on either surface. June 25, 26, 27, 28, 30, July 1, 2, 7 (leaf yellow), 8, no change. |
| 3 | 2 | 2 | 25 | 10 | No conidiophores on either surface. June 25, 26, 27, 28, 30, July 1, 2, 7, 8, no change. |
| 3 | 4 | 1 | July 7 | 10 | No conidiophores on either surface. |
| | | | 8 | 15 | Conidiophores forming only on lower surface. |
| | | | 10 | 62 | Conidiophores abundant above and conidia abundant below. |
| | | | 12 | 100 | Conidia abundant on both surfaces. July 14, no change. |
| | | | 16 | 69 | Conidia very few above and abundant below. |
| | | | 21 | 25 | No conidia on either surface. |
| **3 | 8 | 1 | 7 | 10 | No conidiophores on either surface. July 8, no change. |
| | | | 10 | 25 | Conidiophores abundant on both surfaces. |
| | | | 12 | 100 | Conidia abundant on both surfaces. July 14, 16, 21 (center of spot gone), no change. |
| | | | 23 | 94 | Conidia fairly abundant above and abundant below. |
| | | | 25 | 50 | Conidia few on both surfaces, leaving and leaf yellowing. |
| **3 | 8 | 2 | 7 | 10 | No conidiophores on either surface. July 8, no change. |
| | | | 10 | 25 | Conidiophores abundant on both surfaces. |
| | | | 12 | 100 | Conidia abundant on both surfaces. July 14, no change. |
| | | | 16 | 94 | Conidia fairly abundant above and abundant below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| | | | 25 | 50 | Conidia few on both surfaces. |
| †3 | 8 | 3 | 7 | 10 | No conidiophores on either surface. July 8, no change. |
| | | | 10 | 25 | Conidiophores abundant on both surfaces. |
| | | | 12 | 100 | Conidia abundant on both surfaces. July 14, no change. |
| | | | 16 | 94 | Conidia fairly abundant above and abundant below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| | | | 25 | 75 | Conidia few above and abundant below. |
| 3 | 9 | 1 | 23 | 20 | Conidiophores numerous above and few below. |
| | | | 25 | 44 | Conidia few above and very few below. |
| | | | 28 | 50 | Conidia few and matted together on both surfaces. |
| 4 | 1 | 1 | June 24 | 10 | No conidiophores on either surface. June 25 (leaf dying), 26 (leaf dead), 27, 28, 30, July 1, no change. |
| 4 | 3 | 1 | July 8 | 10 | No conidiophores on either surface. |
| | | | 12 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 14 | 50 | Conidia leaving, few on both surfaces. July 16, 21 (leaf dead), no change. |
| **4 | 5 | 1 | June 30 | 10 | No conidiophores on either surface. July 1, 2, no change. |
| | | | July 7 | 30 | Conidia forming on both surfaces. July 8, no change. |
| | | | 10 | 25 | Only conidiophores on both surfaces. |
| | | | 12 | 100 | Conidia abundant on both surfaces. July 14, no change. |
| | | | 16 | 88 | Conidia fairly abundant on both surfaces. July 21, 23, no change. |
| | | | 25 | 50 | Conidia few on both surfaces. |
| *4 | 12 | 1 | 21 | 50 | Conidia few on both surfaces. July 23, no change. |
| | | | 25 | 76 | Conidia numerous on both surfaces. |
| 5 | 1 | 2 | 1 | 10 | No conidiophores on either surface. July 2, 7, 8, no change. |
| 5 | 2 | 1 | 2 | 10 | No conidiophores on either surface. July 7, no change. |
| | | | 8 | 20 | Conidiophores forming only on upper surface. |
| | | | 10 | 30 | Conidiophores above and conidia forming below. |
| | | | 12 | 50 | Conidia few on both surfaces. |
| | | | 14 | 50 | Conidia very few above, and fairly numerous below. |
| | | | 16 | 50 | Leaf dead, no change in spot. |

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| †5 | 4 | 3 | July 7 | 20 | No conidiophores above, very few conidia forming below. |
| | | | 8 | 37 | Conidiophores on upper surface, few conidia on lower. |
| | | | 10 | 88 | Conidia numerous above and very abundant below. |
| | | | 12 | 75 | Conidia few above and abundant below. July 14, no change. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23 (leaf yellow), July 25, no change. |
| | | | 28 | 63 | Conidia few above and numerous below. |
| 5 | 4 | 5 | 8 | 25 | Conidiophores on both surfaces. |
| | | | 10 | 37 | Conidia few above and conidiophores below. July 12, no change. |
| | | | 14 | 50 | Conidia few on both surfaces. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| | | | 25 | 50 | Conidia few on both surfaces. |
| | | | 28 | 25 | No conidia on either surface. |
| **5 | 6 | 1 | 7 | 17 | Conidiophores none above and forming below. |
| | | | 8 | 25 | Conidiophores on both surfaces. |
| | | | 10 | 75 | Conidia few above and very abundant below. July 12, 14, 16, no change. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| | | | 25 | 57 | Conidia very few above and numerous below. |
| | | | 28 | 37 | Conidia none above and few below. |
| 5 | 8 | 4 | 14 | 10 | No conidiophores on either surface. |
| | | | 16 | 17 | Conidiophores none above and forming below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| | | | 25 | 75 | Conidia abundant above and few below. |
| *5 | 8 | 5 | 23 | 25 | Conodiophores on both surfaces. |
| | | | 25 | 75 | Conidia numerous on both surfaces. |
| 5 | 10 | 1 | 7 | 40 | Conidia very few on either surface but more on lower. July 8, no change. |
| | | | 10 | 75 | Conidia few above and abundant below. |
| | | | 12 | 100 | Conidia abundant on both surfaces. July 14, 16, 21, no change. |
| 5 | 10 | 2 | 7 | 35 | Conidiophores forming above and conidia few below. July 8, no change. |
| | | | 10 | 62 | Conidiophores well developed above and conidia abundant below. |
| | | | 12 | 70 | Conidia very few above and abundant below. |
| | | | 14 | 75 | Conidia few above and abundant below. July 16, no change. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| 5 | 10 | 3 | 14 | 10 | No conidiophores on either surface. |
| | | | 16 | 17 | Conidiophores above and none below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| | | | 25 | 63 | Conidia few above and numerous below. |
| *5 | 12 | 1 | 23 | 25 | Conidiophores on both surfaces. |
| | | | 25 | 70 | Conidia very few above and abundant below. |
| | | | 28 | 63 | Conidia few above and numerous and matted below. |
| 6 | 1 | 1 | June 24 | 10 | No conidiophores on either surface. |
| | | | 25 | 20 | Conidiophores very few above and abundant below. June 26, no change. |
| | | | 27 | 31 | Conidiophores above and conidia very few below. June 28, 30, July 1, 2 (leaf dying), no change. |
| | | | July 7 | 25 | Conidiophores, but no conidia present. |
| 7 | 6 | 1 | 7 | 10 | No conidiophores on either surface. |
| | | | 9 | 56 | Conidia few above and fairly numerous below. |
| | | | 10 | 62 | Conidia none above and abundant below. |
| | | | 12 | 100 | Conidia abundant on both surfaces. July 14, 16, 21, 23, 25, no change. |
| 7 | 6 | 3 | 10 | 15 | Conidiophores forming on upper surface only. |
| | | | 12 | 75 | Conidia abundant above and few below. |
| | | | 14 | 50 | Conidia few on either surface. July 16, no change. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, 25, no change. |
| 7 | 6 | 4 | 16 | 10 | No conidiophores on either surface. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, 25, no change. |

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph. values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| *7 | 6 | 8 | July 21 | 56 | Conidia few above and fairly numerous below. July 23, no change. |
| | | | 25 | 100 | Conidia abundant on both surfaces. |
| *7 | 8 | 5 | 21 | 37 | Conidia very few on both surfaces. July 23, no change. |
| | | | 25 | 75 | Conidia numerous on both surfaces. |
| | | | 28 | 50 | Conidia few on both surfaces. July 30, no change. |
| 7 | 8 | 6 | 21 | 50 | Conidia few on both surfaces. |
| | | | 23 | 56 | Conidia few above and fairly numerous below. |
| | | | 25 | 63 | Conidia numerous above and few below. |
| | | | 28 | 50 | Conidia few on both surfaces. July 30, Aug. 1, no change. |
| *7 | 8 | 7 | July 23 | 15 | Conidiophores none above and few below. |
| | | | 25 | 75 | Conidia few above and abundant below. July 28, 30, no change. |
| | | | Aug. 1 | 50 | Conidia none above and numerous below. |
| 7 | 8 | 12 | July 25 | 25 | Conidiophores on both surfaces. |
| | | | 28 | 45 | Conidia few forming on both surfaces. |
| | | | 30 | 50 | Conidia few on both surfaces. August 1, no change. |
| 7 | 15 | 3 | 28 | 25 | Conidiophores on both surfaces. |
| | | | 30 | 63 | Conidia few above and numerous below. |
| | | | Aug. 1 | 88 | Conidia numerous above and abundant below. |
| †††8 | 8 | 5 | July 9 | 10 | No conidiophores on either surface. |
| | | | 10 | 100 | Conidia abundant on both surfaces. |
| *8 | 12 | 1 | 23 | 50 | Conidia few on both surfaces. |
| | | | 25 | 75 | Conidia numerous on both surfaces. July 28, no change. |
| | | | 30 | 88 | Conidia numerous above and abundant below. August 1, no change. |
| | | | Aug. 5 | 75 | Conidia numerous on both surfaces. Aug. 7, 9, 11, no change. |
| 8 | 23 | 13 | 5 | 15 | Conidiophores few above and none below. Aug. 7, no change. |
| | | | 9 | 25 | Conidiophores on both surfaces. |
| | | | 11 | 57 | Conidia numerous above and very few below. |
| | | | 13 | 100 | Conidia abundant on both surfaces. |
| †8 | 23 | 18 | 7 | 10 | No conidiophores on either surface. Aug. 9, no change. |
| | | | 11 | 60 | Conidia abundant above and conidiophores few below. |
| | | | 13 | 63 | Conidia numerous above and few below. Aug. 15, 18, no change. |
| | | | 22 | 100 | Conidia abundant on both surfaces. |
| †8 | 24 | 1 | July 28 | 75 | Conidia numerous on both surfaces. |
| | | | 30 | 62 | Conidia fairly numerous on both surfaces. Aug. 1, no change. |
| | | | Aug. 5 | 44 | Conidia few above and very few below. |
| | | | 7 | 75 | Conidia numerous and matted above and numerous below. |
| 8 | 25 | 3 | 1 | 10 | No conidiophores on either surface. |
| | | | 5 | 44 | Conidia very few above and few below. |
| | | | 7 | 56 | Conidia few above and fairly numerous below. |
| ***8 | 29 | 1 | 1 | 10 | No conidiophores on either surface. |
| | | | 5 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 7 | 75 | Conidia numerous on both surfaces. |
| | | | 9 | 50 | Conidia few on both surfaces. |
| | | | 11 | 56 | Conidia few above and numerous below. |
| | | | 13 | 100 | Conidia abundant on both surfaces. Aug. 15, no change. |
| | | | 18 | 75 | Conidia few above and abundant below. |
| | | | 22 | 88 | Conidia numerous above and abundant below. Aug. 25, 28, no change. |
| | | | 30 | 63 | Conidia few above and numerous below. Sept. 1, no change except conidia matted above. |
| | | | Sept. 3 | 57 | Conidia very few above and numerous below. |
| 8 | 29 | 3 | Aug. 5 | 25 | Conidiophores on both surfaces. |
| | | | 7 | 30 | Conidiophores above and conidia forming below. |
| | | | 9 | 50 | Conidia very few above and fairly numerous below. |
| | | | 11 | 100 | Conidia abundant on both surfaces. Aug. 13, 15, 18, 22, 25, no change. |

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| ***,†8 | 29 | 6 | Aug. 5 | 20 | Conidiophores very few on both surfaces. Aug. 7, no change. |
| | | | 9 | 75 | Conidia numerous on both surfaces. Aug. 11, no change. |
| | | | 13 | 100 | Conidia abundant on both surfaces. Aug. 15, no change. |
| | | | 18 | 75 | Conidia few above and abundant below. |
| | | | 22 | 69 | Conidia fairly numerous above and numerous below. |
| | | | 25 | 100 | Conidia abundant on both surfaces. |
| | | | 28 | 87 | Conidia fairly abundant on both surfaces. |
| | | | 30 | 75 | Conidia numerous on both surfaces. |
| | | | Sept. 1 | 69 | Conidia fairly numerous above and numerous below. |
| | | | 3 | 62 | Conidia fairly numerous and matted above and fairly numerous below. |
| *** 8 | 29 | 8 | Aug. 5 | 10 | No conidiophores on either surface. Aug. 7, 9, no change. |
| | | | 11 | 24 | Conidia very few above and nothing below. |
| | | | 13 | 88 | Conidia abundant above and numerous below. |
| | | | 15 | 94 | Conidia fairly abundant above and abundant below. |
| | | | 18 | 88 | Conidia numerous above and abundant below. |
| | | | 22 | 100 | Conidia abundant on both surfaces. |
| | | | 25 | 81 | Conidia abundant above and fairly numerous and matted below. |
| 8 | 29 | 9 | 5 | 35 | Condiophores few above and conidia few below. |
| | | | 7 | 50 | Conidiophores numerous above and conidia numerous below. |
| | | | 9 | 88 | Conidia numerous above and abundant below. |
| | | | 11 | 37 | Conidia few above and none below. |
| | | | 13 | 75 | Conidia numerous and matted above and numerous below. Aug. 15, no change. |
| | | | 18 | 88 | Conidia numerous above and abundant below. |
| ***,† 8 | 29 | 11 | 7 | 10 | Conidiophores few on both surfaces. |
| | | | 9 | 44 | Conidia very few above and few below. |
| | | | 13 | 75 | Conidia numerous on both surfaces. Aug. 15, no change. |
| | | | 18 | 88 | Conidia numerous above and abundant below. |
| | | | 22 | 63 | Conidia very few and matted above and fairly abundant below. |
| | | | 25 | 88 | Conidia numerous and matted above and abundant below. |
| | | | 28 | 75 | Conidia few above and abundant below. |
| | | | 30 | 88 | Conidia numerous and matted above and abundant below. |
| | | | Sept. 1 | 82 | Conidia numerous and matted above and fairly abundant below. |
| | | | 3 | 69 | Conidia few and matted above and fairly abundant below. |
| | | | 6 | 50 | Conidia very few and matted above and fairly numerous and matted below. |
| | | | 8 | 43 | Conidia none above and fairly numerous and matted below. |
| §8 | 33 | 1 | 1 | 10 | No conidiophores on either surface. |
| | | | 5 | 81 | Conidia fairly numerous and matted above and abundant below. Aug. 7, no change. |
| | | | 9 | 69 | Conidia fairly numerous and matted above and numerous below. |
| | | | 11 | 63 | Conidia numerous above and few below. |
| | | | 13 | 100 | Conidia abundant on both surfaces. |
| | | | 15 | 81 | Conidia fairly numerous and matted above and abundant below. Aug. 18, no change. |
| | | | 22 | 63 | Conidia few and matted above and numerous below. Aug. 25, no change. |
| | | | 28 | 50 | Conidia none above and numerous below. |
| | | | 30 | 56 | Conidia none above and fairly abundant below. |
| ††8 | 35 | 9 | 13 | 10 | No conidiophores on either surface. |
| | | | 15 | 15 | Conidiophores few above and none below. |
| | | | 18 | 37 | Conidia very few on both surfaces. |
| | | | 22 | 44 | Conidia very few above and few below. |
| | | | 25 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 28 | 69 | Conidia numerous above and fairly numerous below. Aug. 30, Sept. 1, no change.. |
| | | | 3 | 62 | Conidia fairly numerous and matted above and fairly numerous below. |
| | | | 6 | 50 | Conidia few and matted on both surfaces. |
| | | | 8 | 37 | Conidia very few on both surfaces. Sept. 10, no change except matted on both surfaces. |

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| ††,§8 | §35 | 10 | Aug. 13 | 10 | No conidiophores on either surface. |
| | | | 15 | 15 | Conidiophores very few above and none below. August 18, no change. |
| | | | 22 | 37 | Conidia very few on both surfaces. |
| | | | 25 | 56 | Conidia fairly numerous above and few below. |
| | | | 28 | 50 | Conidia fairly numerous above and very few below. |
| | | | 30 | 88 | Conidia numerous and matted above and abundant below. September 1, 3, 6, no change. |
| | | | Sept. 8 | 50 | Conidia few and matted on both surfaces. |
| | | | 10 | 37 | Conidia very few and matted on both surfaces. |
| ††,§8 | §37 | 1 | Aug. 11 | 24 | Conidia very few above and nothing below. |
| | | | 13 | 100 | Conidia abundant on both surfaces. August 15, no change except conidia matted above. |
| | | | 18 | 94 | Conidia fairly abundant and matted above and abundant below. |
| | | | 22 | 88 | Conidia numerous above and abundant below. August 25, no change. |
| | | | 28 | 75 | Conidia numerous on both surfaces. |
| | | | 30 | 69 | Conidia fairly numerous above and numerous below. September 1, 3, no change. |
| | | | Sept. 6 | 37 | Conidia very few on both surfaces. |
| | | | 8 | 25 | Conidia none on either surface. September 10, 13, 15, no change. |
| ††8 | 37 | 2 | Aug. 11 | 10 | No conidiophores on either surface. |
| | | | 13 | 100 | Conidia abundant above and abundant and matted below. |
| | | | 15 | 94 | Conidia abundant above and fairly abundant below. |
| | | | 18 | 87 | Conidia fairly abundant on both surfaces. |
| | | | 22 | 100 | Conidia abundant on both surfaces. August 25, no change except slightly matted on both surfaces. |
| | | | 28 | 94 | Conidia abundant and matted above and fairly abundant below. |
| | | | 30 | 100 | Conidia abundant and matted on both surfaces. |
| | | | Sept. 1 | 87 | Conidia fairly abundant and matted on both surfaces. September 3, 6, no change. |
| | | | 8 | 57 | Conidia very few above and fairly numerous below. September 10, no change. |
| | | | 13 | 37 | Conidia none above and few below. September 15, no change, conidia still matted on both surfaces. |
| ††8 | 37 | 3 | Aug. 11 | 10 | No conidiophores on either surface. |
| | | | 13 | 18 | Conidiophores very few on both surfaces. |
| | | | 15 | 44 | Conidia very few above and few below. |
| | | | 18 | 50 | Conidia very few above and fairly numerous below. |
| | | | 22 | 62 | Conidia fairly numerous and matted above and fairly numerous below. |
| | | | 25 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 28 | 75 | Conidia numerous on both surfaces. August 30, no change, conidia matted on both surfaces. |
| | | | Sept. 1 | 62 | Conidia fairly numerous and matted on both surfaces. |
| §8 | 37 | 6 | Aug. 18 | 10 | No conidiophores on either surface. |
| | | | 22 | 50 | Conidia few on both surfaces. |
| | | | 25 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 28 | 75 | Conidia fairly abundant in center on both surfaces. |
| | | | 30 | 70 | Conidia numerous in center above and fairly abundant in center blow. September 1, no change. |
| | | | Sept. 3 | 69 | Conidia fairly numerous above and fairly abundant in center below. |
| | | | 6 | 87 | Conidia fairly abundant on both surfaces. |
| | | | 8 | 75 | Conidia fairly numerous and matted above and fairly abundant below. September 10, no change. |
| | | | 13 | 94 | Conidia fairly abundant above and abundant below. September 15, no change. |
| | | | 17 | 50 | Conidia few on either surface. |
| 8 | 38 | 1 | Aug. 11 | 10 | No conidiophores on either surface. |
| | | | 13 | 69 | Conidia numerous above and fairly numerous below. |
| | | | 15 | 69 | Conidia fairly numerous above and numerous and matted below. |
| | | | 18 | 82 | Conidia fairly abundant above and numerous and matted below. |
| | | | 22 | 69 | Conidia numerous and matted above and fairly numerous below. |
| 8 | 39 | 6 | Aug. 18 | 10 | No conidiophores on either surface. |
| | | | 22 | 63 | Conidia numerous above and few below. |
| | | | 25 | 82 | Conidia fairly abundant above and numerous below. |

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| 8 | 41 | 3 | Aug. 22 | 31 | Conidiophores above and conidia very few below. |
| | | | 25 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 28 | 82 | Conidia numerous above and fairly abundant below. |
| | | | 30 | 88 | Conidia numerous above and abundant below. September 1, no change. |
| | | | Sept. 3 | 74 | Conidia fairly numerous above and fairly abundant below. |
| | | | 6 | 69 | Conidia fairly numerous and matted above and numerous and matted below. September 8, 10, no change. |
| | | | 13 | 57 | Conidia very few and matted above and numerous below. September 15, 17, no change. |
| | | | 19 | 50 | Conidia none above and numerous below. |
| ††, §8 | 44 | 1 | Aug. 25 | 10 | No conidiophores on either surface. |
| | | | 28 | 24 | Conidia very few above and nothing below. |
| | | | 30 | 44 | Conidia few above and very few below. September 1, 3, 6, no change. |
| | | | Sept. 8 | 50 | Conidia few on both surfaces. |
| | | | 10 | 56 | Conidia fairly numerous above and few below. |
| | | | 13 | 44 | Conidia very few above and few below. |
| | | | 15 | 69 | Conidia fairly numerous and matted above and numerous below. |
| | | | 17 | 44 | Conidia very few and matted above and few below. |
| | | | 19 | 31 | Conidia none above and very few below. |
| ††8 | 44 | 2 | Aug. 25 | 15 | Conidiophores forming above and nothing below. |
| | | | 28 | 75 | Conidia fairly abundant in center of both surfaces. |
| | | | 30 | 90 | Conidia abundant in center of both surfaces. September 1, 3, 6, no change. |
| | | | Sept. 8 | 69 | Conidia fairly numerous above and numerous below. |
| | | | 10 | 100 | Conidia abundant on both surfaces. September 13, 15, no change. |
| | | | 17 | 56 | Conidia few and matted above and fairly numerous and matted below. |
| | | | 19 | 50 | Conidia very few and matted above and fairly numerous and matted below. |
| 8 | 44 | 3 | Aug. 25 | 10 | No conidiophores on either surface. |
| | | | 28 | 80 | Conidia fairly abundant in center on both surfaces. |
| | | | 30 | 90 | Conidia fairly abundant in center above and abundant in center below. September 1, 3, no change. |
| | | | Sept. 6 | 87 | Conidia fairly abundant and matted above and fairly abundant below. September 8, no change. |
| | | | 10 | 94 | Conidia fairly abundant above and abundant below. September 13, no change. |
| | | | 15 | 88 | Conidia numerous and matted above and abundant below. |
| | | | 17 | 75 | Conidia fairly numerous and matted above and fairly abundant below. |
| | | | 19 | 63 | Conidia few and matted above and numerous below. |
| ††, §8 | 45 | 1 | Aug. 22 | 37 | Conidia very few on both surfaces. |
| | | | 25 | 56 | Conidia few above and fairly numerous and matted below. |
| | | | 28 | 82 | Conidia numerous and matted above and fairly abundant below. |
| | | | 30 | 88 | Conidia numerous above and abundant below. September 1, no change. |
| | | | Sept. 3 | 81 | Conidia fairly numerous and matted above and abundant below. September 6, 8, no change except conidia matted below. |
| | | | 10 | 75 | Conidia fairly numerous and matted above and fairly abundant and matted below. |
| | | | 13 | 69 | Conidia few and matted above and fairly abundant and matted below. |
| | | | 15 | 50 | Conidia none above and numerous and matted below. |
| | | | 17 | 31 | Conidia none above and very few and matted below. |
| | | | 19 | 25 | Conidia none on either surface. |
| 8 | 45 | 2 | Aug. 25 | 10 | No conidiophores on either surface. |
| | | | 28 | 35 | Conidia few above and conidiophores forming below. |
| | | | 30 | 25 | Conidia very few above and nothing below. |
| | | | Sept. 1 | 29 | Conidia very few above and few conidiophores below. September 3, no change. |
| | | | 6 | 44 | Conidia few above and very few below. |
| | | | 8 | 50 | Conidia few and matted above and few below. |
| | | | 10 | 44 | Conidia few and matted above and very few below. September 13, 15, 17, no change. |
| | | | 19 | 25 | Conidia none on either surface. |

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| 8 | 45 | 5 | Aug. 25 | 10 | No conidiophores on either surface. |
| | | | 28 | 56 | Conidia few above and fairly numerous below. August 30, September 1, 3, no change. |
| | | | Sept. 6 | 65 | Conidia few and matted above and fairly abundant in center below. |
| | | | 8 | 58 | Conidia few and matted above and numerous in center below. |
| | | | 10 | 69 | Conidia few and matted above and fairly abundant below. |
| | | | 13 | 63 | Conidia few and matted above and numerous in center below. September 15, no change. |
| 8 | 49 | 1 | Aug. 28 | 37 | Conidia very few on both surfaces. |
| | | | 30 | 50 | Conidia few on both surfaces. September 1, 3, no change. |
| | | | Sept. 6 | 69 | Conidia numerous above and fairly numerous below. |
| | | | 8 | 62 | Conidia fairly numerous and matted above and fairly numerous below. |
| | | | 10 | 75 | Conidia numerous and slightly matted on both surfaces. |
| | | | 13 | 56 | Conidia fairly numerous and matted above and few below. |
| 8 | 49 | 2 | Aug. 28 | 10 | No conidiophores on either surface. |
| | | | 30 | 25 | No conidiophores above and conidia very few below, September 1, 3, no change. |
| | | | Sept. 6 | 56 | Conidia few above and fairly numerous below. |
| | | | 8 | 50 | Conidia very few above and fairly numerous below. September 10, 13, no change. |
| 8 | 49 | 3 | Aug. 28 | 10 | No conidiophores on either surface. |
| | | | 30 | 15 | Conidiophores few above and very few below. |
| | | | Sept. 1 | 30 | Conidiophores few above and conidia very few below. September 3, no change. |
| | | | 6 | 62 | Conidia fairly numerous on both surfaces. |
| | | | 8 | 65 | Conidia fairly numerous above and numerous in center below. |
| | | | 10 | 75 | Conidia numerous above and numerous and matted in center below. |
| | | | 13 | 62 | Conidia fairly numerous on both surfaces. |
| 8 | 49 | 6 | 1 | 10 | No conidiophores upon either surface. September 3, no change. |
| | | | 6 | 44 | Conidia very few above and few below. September 8, no change. |
| | | | 10 | 50 | Conidia few on both surfaces. September 13, no change. |
| 8 | 49 | 7 | 1 | 10 | No conidiophores on either surface. September 3, no change. |
| | | | 6 | 24 | Nothing above and conidia very few below. |
| | | | 8 | 44 | Conidia very few above and few below. September 10, 13, no change. |
| 8 | 49 | 8 | 1 | 10 | No conidiophores on either surface. September 3, 6, no change. |
| | | | 8 | 20 | Nothing above and conidia forming below. |
| | | | 10 | 44 | Conidia very few above and few below. September 13, no change. |
| 8 | 49 | 9 | 3 | 10 | No conidiophores on either surface. September 6, no change. |
| | | | 8 | 20 | Nothing above and very few conidia forming below. |
| | | | 10 | 44 | Conidia very few above and few below. September 13, no change. |
| 8 | 49 | 10 | 3 | 10 | No conidiophores on either surface. September 6, 8, no change. |
| | | | 10 | 37 | Conidia very few on both surfaces. September 13, no change. |
| 8 | 51 | 1 | 3 | 10 | No conidiophores on either surface. September 6, no change. |
| | | | 8 | 24 | Conidia very few above and nothing below. September 10, no change. |
| | | | 13 | 37 | Conidia very few upon both surfaces. September 15, 17, no change. |
| 8 | 51 | 2 | 6 | 10 | No conidiophores on either surface. |
| | | | 8 | 37 | Conidia very few on both surfaces. |
| | | | 10 | 65 | Conidia numerous in the center upon both surfaces. September 13, 15, no change. |
| | | | 17 | 44 | Conidia very few above and few below. |
| 8 | 51 | 3 | 6 | 10 | No conidiophores on either surface. September 8, no change. |
| | | | 10 | 37 | Conidia very few on both surfaces. |
| | | | 13 | 44 | Conidia few above and very few below. September 15, 17, no change. |

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| 8 | 51 | 4 | Sept. 6 | 10 | No conidiophores on either surface. September 8, 10, no change. |
| | | | 13 | 30 | Conidiophores forming above and conidia very few below. September 15, 17, no change. |
| 8 | 51 | 5 | 6 | 10 | No conidiophores on either surface. September 8, 10, 13, 15, 17, no change. |
| 8 | 51 | 6 | 6 | 10 | No conidiophores on either surface. September 8, 10, 13, no change. |
| | | | 15 | 15 | Conidiophores none above and forming below. |
| | | | 17 | 24 | Nothing above and very few conidia below. |
| 8 | 53 | 1 | 17 | 10 | No conidiophores on either surface. September 19, no change. |
| 8 | 53 | 2 | 17 | 10 | No conidiophores on either surface. September 19, no change. |
| 9 | 3 | 1 | June 24 | 10 | No conidiophores on either surface. June 25, 26, 27, 28, 30; July 1, 2, 7, no change. |
| **9 | 3 | 2 | July 9 | 10 | No conidiophores on either surface. |
| | | | 10 | 25 | Conidiophores abundant on both surfaces. |
| | | | 12 | 87 | Conidia fairly abundant on both surfaces. July 14, no change. |
| | | | 16 | 100 | Conidia abundant on both surfaces. |
| | | | 21 | 37 | Leaf dead, conidia very few on both surfaces. July 25, 28, no change. |
| 9 | 5 | 1 | 21 | 50 | Conidia few on both surfaces. July 23, 25, no change. |
| | | | 28 | 37 | Conidia none above and few below. July 30, no change. |
| *9 | 5 | 2 | 23 | 17 | Conidiophores above and none below. |
| | | | 25 | 75 | Conidia numerous on both surfaces. July 28, 30, no change. |
| 9 | 7 | 1 | 9 | 10 | No conidiophores on either surface. |
| | | | 10 | 37 | Conidia few above and conidiophores abundant below. |
| | | | 12 | 94 | Conidia abundant above and fairly abundant below. July 14, 16, no change. |
| 10 | 3 | 1 | June 30 | 10 | No conidiophores on either surface. July 1, 2, 7, 9, 10, 12, no change. |
| 10 | 5 | 1 | July 2 | 10 | No conidiophores on either surface. |
| | | | 7 | 20 | Conidiophores few above and abundant below. |
| | | | 9 | 37 | Conidiophores above and conidia few below. |
| | | | 10 | 75 | Conidia numerous on both surfaces. July 12, 14, no change. |
| | | | 16 | 100 | Conidia abundant on both surfaces. July 21, 23, no change. |
| | | | 25 | 75 | Conidia few above and abundant below. |
| | | | 28 | 50 | Conidia none above and numerous below. Leaf dead. July 30, no change. |
| 10 | 5 | 3 | 21 | 50 | Conidia few on both surfaces. July 23, no change. |
| | | | 25 | 56 | Conidia fairly numerous above and few below. |
| | | | 28 | 25 | Conidia none on either surface. July 30, no change. |
| 10 | 5 | 4 | 21 | 37 | Conidiophores above and conidia few below. |
| | | | 23 | 44 | Conidia very few above and few below. |
| | | | 25 | 37 | Conidia very few on both surfaces. |
| | | | 28 | 25 | Conidia none on either surface. July 30, no change. |
| 10 | 6 | 1 | 2 | 10 | No conidiophores on either surface. |
| | | | 7 | 31 | Conidia very few above and conidiophores below. |
| | | | 9 | 62 | Conidia fairly numerous on both surfaces. July 10, 12, 14, no change. |
| | | | 16 | 56 | Conidia few above and fairly numerous below. |
| 10 | 6 | 6 | July 12 | 10 | No conidiophores on either surface. |
| | | | 14 | 15 | Conidiophores none above and very few below. |
| | | | 16 | 37 | Conidiophores above and conidia few below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, 25, 28, 30, no change. |

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

| Plant No. | Leaf No. | Spot No. | Date. | Graph values. | Data on life histories. |
|---|---|---|---|---|---|
| | | | 1913. | | |
| **10 | 9 | 1 | July 7 | 50 | Conidia few on both surfaces. July 9, no change. |
| | | | 10 | 75 | Conidia few above and abundant below. |
| | | | 12 | 94 | Conidia fairly abundant above and abundant below. July 14, no change. |
| | | | 16 | 69 | Conidia very few above and abundant below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, no change. |
| | | | 25 | 75 | Conidia numerous on both surfaces. |
| †10 | 9 | 2 | 7 | 37 | Conidiophores above and conidia few below. July 9, no change. |
| | | | 10 | 75 | Conidia few above and abundant below. |
| | | | 12 | 69 | Conidia few above and fairly abundant below. July 14, no change. |
| | | | 16 | 69 | Conidia very few above and abundant below. |
| | | | 21 | 100 | Conidia abundant on both surfaces. July 23, 25, no change. |
| 10 | 9 | 3 | 23 | 25 | Conidiophores on both surfaces. |
| | | | 25 | 75 | Conidia numerous on both surfaces. July 28, no change. |
| *10 | 10 | 1 | 23 | 10 | No conidiophores on either surface. |
| | | | 25 | 75 | Conidia numerous on both surfaces. |
| | | | 28 | 75 | Conidia abundant above and few and matted below. July 30, no change. |
| 10 | 11 | 1 | 25 | 75 | Conidia numerous on both surfaces. |
| | | | 28 | 75 | Conidia abundant above and few and matted below. July 30, no change. |
| 10 | 11 | 3 | 25 | 50 | Conidiophores above and conidia numerous below. |
| | | | 28 | 75 | Conidia numerous on both surfaces. July 30, no change. |

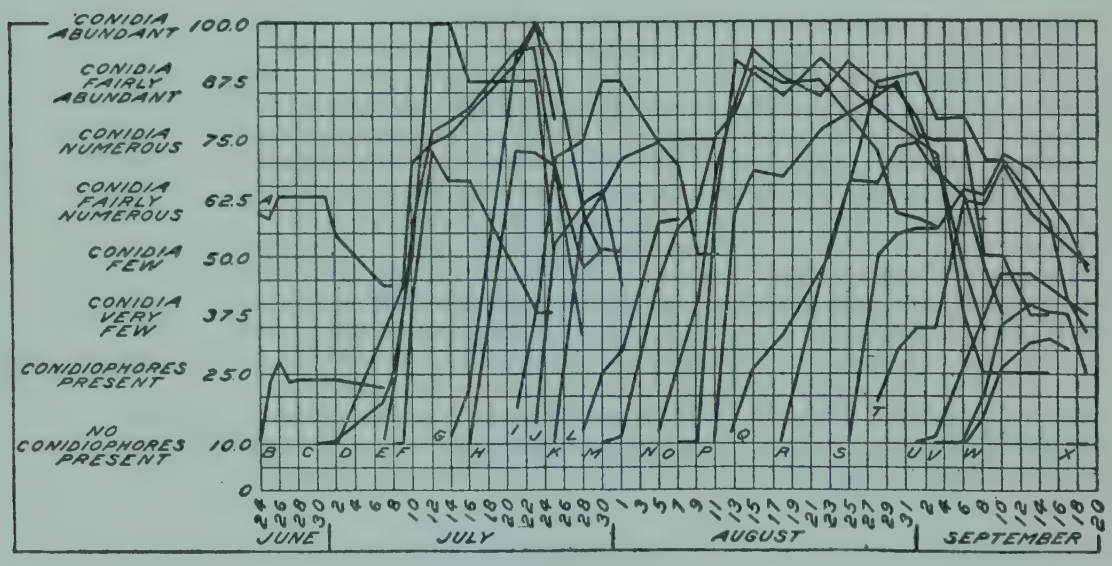


FIG. 6.—Curves of the leaf spot history series, showing the production of conidia on different dates from June 24 to September 19, 1913, at Rocky Ford, Colo.

After the values were all assigned, the spots which appeared on or about the same day were brought together in 24 groups and averaged (Table V and fig. 6). The temperature and humidity records used in the correlations with the leafspot histories were taken among the sugar-beet leaves near the surface of the ground.

TABLE V.—*Leafspot history series showing their arbitrary values on different dates and the number of spots entering into the average of each series at Rocky Ford, Colo., 1913*

| Date. | Series and values. | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X |
| June 24 | 59 | 11 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 25 | 57.5 | 23 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 26 | 62.5 | 27.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 27 | 62.5 | 23 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 28 | 62.5 | 23.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 30 | 62.5 | 23.5 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| July 1 | 62.5 | 23.5 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 2 | 54 | 23.5 | 10 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 7 | 43.5 | 21.5 | 19 | 34 | 11 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 8 | 43.5 | ..... | 24 | ..... | 20.5 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 9 | ..... | ..... | ..... | 43 | ..... | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 10 | ..... | ..... | 50 | 70 | 56 | 41 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 12 | ..... | ..... | 100 | 74 | 77 | 73 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 14 | ..... | ..... | 100 | 76 | 79 | 66 | 11 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 16 | ..... | ..... | 87.5 | 80.5 | 81.5 | 66 | 21 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 21 | ..... | ..... | 87.5 | 91 | 94 | 46 | 93.5 | 72.5 | 17.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 23 | ..... | ..... | 87.5 | 100 | 94.5 | 37.5 | 100 | 72 | 37.5 | 14 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 25 | ..... | ..... | 50 | 91.5 | 69 | 37.5 | 79 | 69 | 71 | 52 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 28 | ..... | ..... | ..... | 58.5 | 33 | ..... | ..... | 47 | 75 | 61 | 58.5 | 12.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 30 | ..... | ..... | ..... | 50 | ..... | ..... | ..... | 51 | 87.5 | 64 | 63 | 25 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Aug. 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 50.5 | 87.5 | 43.5 | 70.5 | 29.5 | 11.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 75 | ..... | 75 | 57 | 45 | 12.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 7 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 75 | ..... | 69.5 | 57.5 | 55.5 | 26 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 75 | ..... | 50 | ..... | 60.5 | 39 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 11 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 75 | ..... | 50 | ..... | 75.5 | 66.5 | 61.5 | 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 13 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 80.5 | 82 | 92 | 58.5 | 12 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 15 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 90.5 | 94 | 89 | 68 | 25.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 18 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 87 | 88 | 84.5 | 67 | 33 | 10.5 | ..... | ..... | ..... | ..... | ..... | ..... |
| 22 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 87.5 | 84 | 92.5 | 77 | 46.5 | 44.5 | ..... | ..... | ..... | ..... | ..... | ..... |
| 25 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 79.5 | 91.5 | 86 | 81 | 65.5 | 65.5 | 10.5 | ..... | ..... | ..... | ..... | ..... |
| 28 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 72 | 86 | 81 | 84 | 65.5 | 87.5 | 50.5 | 19 | ..... | ..... | ..... | ..... |
| 30 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 59 | 86 | 78 | 87.5 | 73.5 | 88 | 54.5 | 29.5 | ..... | ..... | ..... | ..... |
| Sept. 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 58 | 79.5 | 75 | 75 | 75 | 89.5 | 55.5 | 34.5 | 10 | ..... | ..... | ..... |
| 3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 56 | 68.5 | 71.5 | 75 | 68.5 | 79.5 | 55.5 | 34.5 | 10 | 10 | ..... | ..... |
| 6 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 47 | 37.5 | 75 | 62.5 | 79.5 | 64 | 62.5 | 26 | 10 | 10 | ..... |
| 8 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 34 | 25 | 50 | 48 | 70.5 | 63 | 61 | 36 | 19 | 15.5 | ..... |
| 10 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 25 | 50 | 37.5 | 70.5 | 71.5 | 70 | 46 | 35 | 26 | ..... |
| 13 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 25 | 37.5 | ..... | 62.5 | 68.5 | 59 | 46 | 39.5 | 31 | ..... |
| 15 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 25 | 37.5 | ..... | 57.5 | 61.5 | ..... | ..... | 37.5 | 32 | ..... |
| 17 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 40.5 | 56 | ..... | ..... | 37.5 | 30 | 10 |
| 19 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 33.5 | 46.5 | 48 | 37.5 | 25 | ..... | 10 |
| Average number of spots | 4 | 6 | 6 | 29 | 28 | 7 | 4 | 22 | 8 | 18 | 24 | 9 | 72 | 13 | 6 | 7 | 18 | 14 | 9 | 4 | 3 | 3 | 5 | 5 |

CONIDIAL PRODUCTION.—Under favorable conditions conidia are produced apparently much more readily by young than by old leaf-spots. For instance, as will be seen in Table IV, leaf spots (*)[1] 2 to 4 days old showed a marked increase in conidial production from July 23 to 25, while during the same period spots (**) 14 to 24 days old in most cases showed a decrease. However, spots (***) 2 to 3 weeks old on green leaves showed an increased production from August 18 to 25, the conditions being favorable, and some (†) even produced a second and third crop, although usually but one crop (††) is produced and this while the spots are comparatively young. It was also found that under favorable conditions a spot (†††) may produce abundant conidia on both surfaces in one day. Usually the maximum production is reached within 10 days after the spots appear (fig. 6), and sometimes under very favorable conditions the production may increase after this period (fig. 6, curves D and E, July 17 to 23), but the older spots do not always respond to favorable conditions in this way (fig. 6, curves C and F). In no case was a new growth of conidia observed on spots on yellow or dying leaves on green plants in the field. The fungus seemed to lose its vigor much sooner on such leaves than on green leaves which remained attached to the crown at harvest time. From the standpoint of control of the disease this is a very important point, from the fact that at harvest time the green leaves, on which the fungus is vigorous, are removed with the crowns and stored in the silo, while the yellow and dying leaves, on which the fungus may be too weak to overwinter, break off and remain on the ground.

During the greater part of August and September, when the precipitation was light (fig. 7), many of the conidia had a shrunken appearance and were massed together on the leafspot areas (Table IV,§). When placed in water, these conidia did not germinate; consequently this desiccation of the conidia may also be an important factor in connection with the vitality of the fungus on the host.

The position of the leaf on which the spot studied was located was also found to be an important factor in conidial production, an abundance of conidia being frequently observed on leaves protected from the sun, while at the same time few were observed on those exposed to the sun the greater part of the day. This difference in production is thought to be due mainly to the difference in humidity of the protected and the exposed locations.

A study of the comparative production of conidia on the upper and the lower surfaces of the spots was also made, the conidia on the spots included in series E, K, N, and S (fig. 6) being tabulated for this purpose.

Generally a more abundant conidial production was found on the lower than on the upper surface (fig. 8), and this was due apparently to

[1] The asterisks (*), daggers (†), and section marks (§) refer to particular leaf spots in Table IV.

the probably higher humidity of the former. Only during a very favorable period (fig. 7, July 19 to 21) or where the leaves were turned up or protected by other leaves was the conidial production on the upper surface equal to that on the lower surface (fig. 8, series E, July 21). At times, conidia were formed more abundantly on the upper surface than on the lower (fig. 6, series N, August 11, and series S, August 28). Because of the spongy parenchyma and the greater number of stomata on the lower surface, it might be supposed that conidiophores could be produced more readily on this than on the upper surface; but, as above indicated, humidity would seem to be the controlling factor in this connection.

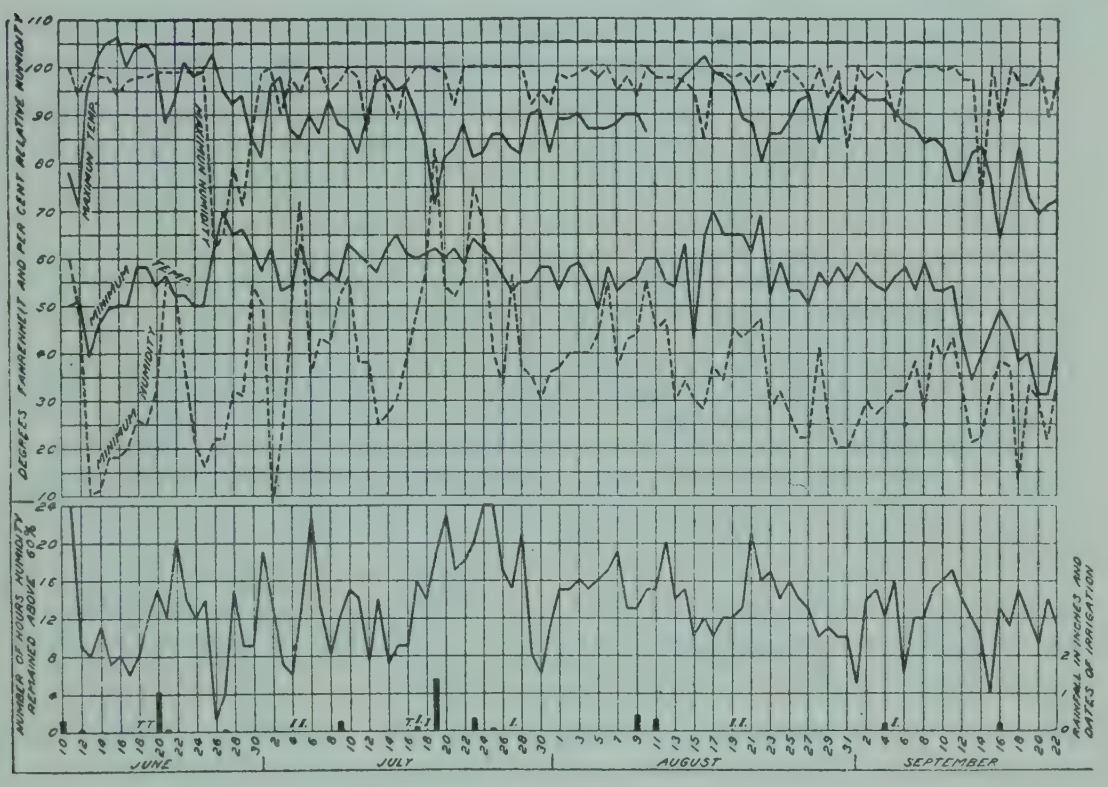


FIG. 7.—Curves of the maximum and minimum temperatures and humidities, the number of hours that the humidity remained above 60 from noon of the preceding to noon of the given day among the plants, and rainfall and irrigation records, taken in a medium-early sugar-beet field from June 10 to September 22, 1913, at Rocky Ford, Colo.

A comparison of the conidial production as shown in Table IV and figure 6 and the climatic data shown in figure 7 indicates many definite relations. When the spots were first found, on June 20 and 24, conidia were fairly numerous (fig. 6, curve A) on all except six spots, which had evidently just developed on the latter date, as no conidia were present at this time and conidiophores only were produced the next two weeks. The following week there was but little increase, and during the next few days many of the conidia were disseminated. The small production of conidia was evidently due directly to the high temperature and the low humidity which prevailed during this period (fig. 7), as conidia were produced in great abundance from July 9 to 12 (fig. 6, curves C, D, E, F), when the temperature was lower and the humidity higher (fig. 7). Dur-

ing this time and the few days just preceding, the humidity remained above 60 for a longer time on an average and the minimum humidity did not become so excessively low nor the temperature so excessively high as during the time previous to July 4.

The next period of pronounced increase in conidial production was from July 19 to 23 (fig. 6, curves D, E, G), when the conditions were more favorable than during any period of similar length through the summer, the humidity ranging above 60 on an average of 19.4 hours each day and not falling below 52 (fig. 7), and the temperature ranging from 60° to 90° F.

Conidial production was again above the average (curves M, N, and O) from August 9 to 13, during which period the humidity remained above 60 from 13 to 20 hours each day and there was a small amount of rain which seemed to aid in maintaining the necessary humid conditions. Production was checked on August 16, on which date the temperature was 102° and the average humidity low, and was again inhibited after

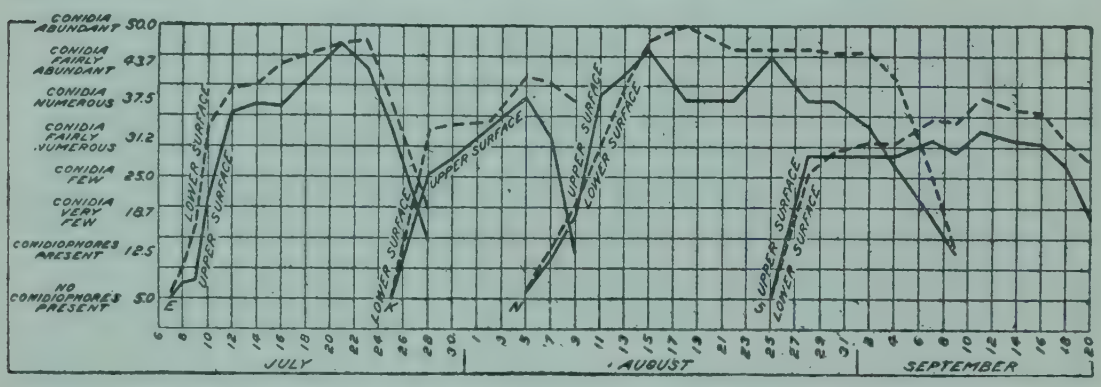


FIG. 8.—Curves of the comparative production of conidia on the upper and lower surfaces of the leaf spots, representing series E, K, N, and G of Table V and figure 6. Rocky Ford, Colo., 1913.

September 11, subsequent to which date the minimum temperatures ranged from about 30° to 45° and the maximum from about 65° to 83°, while the humidity remained above 60 for 12.4 hours per day, on an average.

The general conclusion from these tests is that conidial production is greatly influenced by temperature and relative humidity, or speaking specifically—

(1) A temperature of 100° F. or over is detrimental to conidial production, directly perhaps because it is inimical to the growth of the fungus and indirectly because humidity is ordinarily excessively low at such an extreme temperature.

(2) Conidial production is greatly checked at daily temperatures ranging below 50° as a minimum and 80° as a maximum.

(3) The most favorable temperature for conidial production is 80° to 90° in the daytime and not below 60° at night.

(4) The temperature being favorable, the largest conidial production occurred at the higher humidities. A good production occurred when

the humidity remained above 60 for not less than 15 to 18 hours, but very few were produced when the humidity remained above 60 for less than 10 to 12 hours daily.

With a view to determining the approximate number of conidia produced on a sugar-beet plant under a favorable temperature and humidity, one representing a heavy infection in August was selected. After the infected leaves were measured a representative portion of conidia were carefully washed off into sterilized water and counted. The count, which was made by means of a dilution method, showed 250,000,000 conidia on the plant at that time.

CONIDIAL DISSEMINATION.—That a period of low humidity, with its accompanying factors, is favorable to the dissemination of conidia was frequently observed (fig. 6, curve R). For instance, it was found that the amount of conidia diminished on September 1, 6, 14, and 15, when the humidity remained above 60 for 5, 6, 10, and 4 hours, respectively; while, on the other hand, there was no diminution in the amount present on September 3 to 5 and 8 to 10, during which periods the humidity remained above 60 for 12 to 16 hours.

Rainfall is also an important factor in the dissemination of conidia, as was noted in several instances. On July 19 (fig. 6, curve F) rain fell, and as a result many conidia were washed off, and the same was true in the case of rains on July 23 (curves C, D, E, G, H), August 9 (curve K), September 4 (curves N, O, R), and September 16 (curves Q, S, T, U, V, W). After rains on July 19, August 9, and September 4, however, there were more conidia present than before, but this was probably due to the fact that more were produced under the favorable humid conditions attending these rains than were washed off. It was also found that the conidia were disseminated more rapidly from the upper than from the lower surface of the spots (fig. 8). This was due probably to the greater exposure of the former to wind and rainfall.

## RELATION OF INFECTION CYCLES TO CLIMATIC CONDITIONS

For the purpose of determining the relation of infection cycles to climatic conditions, a study was made of the increase and spread of disease in a field of sugar beets planted about May 1 [1] at Rocky Ford and one planted about two weeks later. Both fields had been in beets for two or three years, and as very few, if any, of the tops were removed after the harvest of 1912, infection appeared early in 1913 and was generally distributed.

Three plants in the early field (Table VII) and ten in the medium-early (Table VI) were selected, the leaves tagged and numbered consecutively, beginning with the outermost or oldest and continuing with the new ones as they appeared. The spots on each leaf were counted at

[1] Conidial production and dissemination were also studied in this field.

frequent intervals,[1] and the average actual increase of spots per plant computed (Table VIII and fig. 9). It was found that from 400 to 1,000 spots on a leaf, depending on its size, killed it within a few days.

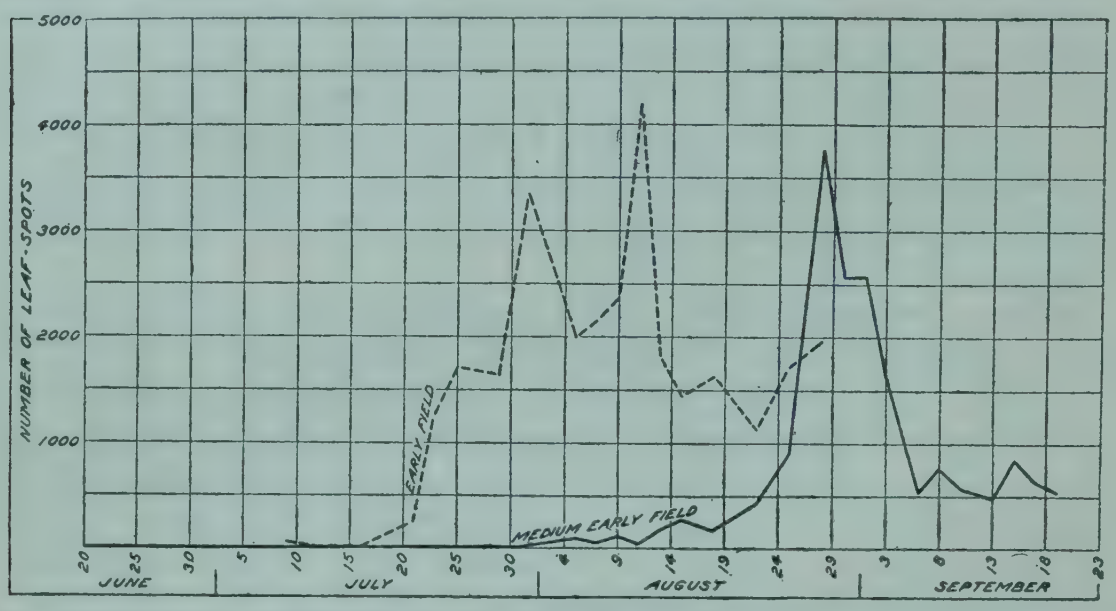


FIG. 9.—Curves of the 2-day average increases in the number of leaf spots per plant in a medium-early and an early sugar-beet field, from June 18 to September 19, 1913, at Rocky Ford, Colo.

TABLE VI.—*Average infection cycle of Cercospora beticola in a medium-early sugar-beet field with poorly developed foliage and with a consequent low humidity early in the season at Rocky Ford, Colo., in 1913*

| Date. | Total number of leaves marked. | Total number of leaves infected. | Total number of leaves dead. | Number of leaves killed by *Cercospora beticola*. | Number of infected green and dying leaves. | Number of functional leaves— | | Total number of functional leaves. | Total number of leaf spots per plant. | Average number of leaf spots per leaf. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Infected. | Uninfected. | | | |
| July 2 | 14.2 | 3.2 | 3.3 | ..... | 3 | 2.9 | 8 | 10.9 | 5 | 1.6 |
| 7 | 16.8 | 4.7 | 3.8 | ..... | 4.4 | 4.1 | 8.9 | 13 | 10.4 | 2.3 |
| 8 | 17.4 | 5.4 | 4.3 | ..... | 4.7 | 4.4 | 8.7 | 13.1 | 11.4 | 2.4 |
| 10 | 18.5 | 5.5 | 4.6 | ..... | 4.5 | 4.3 | 9.6 | 13.9 | 11.2 | 2.4 |
| 12 | 19.7 | 5.5 | 5.1 | ..... | 4.3 | 4 | 10.6 | 14.6 | 10.8 | 2.5 |
| 14 | 21.3 | 5.8 | 5.5 | ..... | 4.3 | 4 | 11.8 | 15.8 | 10.8 | 2.5 |
| 16 | 22.6 | 6 | 6.1 | ..... | 4.1 | 3.8 | 12.7 | 16.5 | 10.4 | 2.5 |
| 21 | 26.3 | 6.6 | 7.1 | ..... | 4.4 | 3.5 | 15.7 | 19.2 | 13.3 | 3 |
| 23 | 27.5 | 7.8 | 8 | ..... | 4.7 | 4.1 | 15.4 | 19.5 | 17.2 | 3.6 |
| 25 | 29 | 8.1 | 8.9 | ..... | 4.4 | 3.5 | 16.6 | 20.1 | 17.5 | 3.9 |
| 28 | 30.5 | 10.8 | 9.3 | ..... | 6.3 | 5.8 | 15.4 | 21.2 | 20.5 | 3.2 |
| 30 | 33 | 17.6 | 11 | ..... | 10.7 | 10.3 | 11.7 | 22 | 42 | 3.9 |
| Aug. 1 | 34 | 27 | 12 | ..... | 20 | 19 | 3 | 22 | 93.5 | 4.7 |
| 5 | 35.5 | 27 | 12.5 | ..... | 19 | 18.5 | 4.5 | 23 | 292 | 15.3 |
| 7 | 36.5 | 27 | 13.5 | ..... | 18.5 | 17.5 | 5.5 | 23 | 355 | 19.1 |
| 9 | 37.5 | 29 | 13.5 | ..... | 19.5 | 19.5 | 4.5 | 24 | 402 | 20.6 |
| 11 | 38.5 | 30 | 15.5 | ..... | 20.5 | 19 | 4 | 23 | 455.5 | 22.2 |
| 13 | 40 | 31 | 15.5 | ..... | 20 | 20 | 4.5 | 24.5 | 593 | 29.6 |
| 15 | 41.5 | 31.5 | 15.5 | ..... | 20.5 | 20.5 | 5.5 | 26 | 875 | 42.6 |
| 18 | 43 | 33.5 | 15.5 | ..... | 22.5 | 22.5 | 5 | 27.5 | 1,155.5 | 51.3 |
| 22 | 45 | 35.5 | 15.5 | ..... | 24.5 | 24.5 | 5 | 29.5 | 2,045.5 | 85.9 |
| 25 | 46 | 38 | 15.5 | ..... | 27 | 27 | 3.5 | 30.5 | 3,420 | 126.6 |
| 28 | 48 | 41 | 17 | 1.5 | 29.5 | 28 | 3 | 31 | 9,081.5 | 307.8 |
| 30 | 48.5 | 41 | 18 | 2 | 28.5 | 27.5 | 3 | 30.5 | 10,856 | 380.9 |
| Sept. 3 | 50 | 41.5 | 23.5 | 6 | 25.5 | 22.5 | 4 | 26.5 | 12,371.5 | 485.1 |
| 6 | 50.5 | 42.5 | 25.5 | 8 | 23.5 | 21.5 | 4 | 25 | 11,493 | 489 |
| 8 | 51.5 | 42.5 | 26.5 | 9 | 21.5 | 20.5 | 5 | 25 | 10,928.5 | 508.3 |
| 10 | 52.5 | 44.5 | 28.5 | 11 | 22.5 | 20.5 | 4.5 | 24 | 10,782 | 479.2 |
| 13 | 53 | 44.5 | 30 | 12.5 | 20.5 | 19 | 4 | 23 | 10,003.5 | 487.9 |
| 15 | 53.5 | 44.5 | 32.5 | 15 | 19 | 16.5 | 4.5 | 21 | 9,585.5 | 504.5 |
| 17 | 54 | 45.5 | 34.5 | 17 | 17.5 | 15.5 | 4 | 19.5 | 8,504. | 485.9 |
| 19 | 55 | 45.5 | 35 | 17.5 | 15.5 | 15 | 5 | 20 | 7,597.5 | 490.1 |

[1] For convenience and uniformity, 2-day averages were used in making the comparisons, the counts being made usually at 2-day intervals.

TABLE VII.—*Average infection cycle of Cercospora beticola in an early sugar-beet field in which there was a heavy production of foliage and a consequent high humidity early in the season at Rocky Ford, Colo., in 1913*

| Date. | Total number of leaves marked. | Total number of leaves infected. | Total number of leaves dead. | Number of leaves killed by *Cercospora beticola*. | Number of infected green and dying leaves. | Number of functional leaves— | | Total number functional leaves. | Total number of leaf spots per plant. | Average number of leaf-spots per leaf. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Infected. | Uninfected. | | | |
| July 7 | 23 | 13.5 | 4 | ..... | 12 | 12 | 7 | 19 | 529 | 44 |
| 9 | 24 | 13.5 | 4.5 | ..... | 12 | 11.5 | 8 | 19.5 | 596.5 | 49.7 |
| 12 | 26 | 14 | 5.5 | ..... | 12 | 11 | 9 | 20.5 | 615 | 51.2 |
| 14 | 27.5 | 14.5 | 5.5 | ..... | 11.5 | 11.5 | 10.5 | 22 | 554.5 | 48.2 |
| 16 | 29.5 | 16 | 7 | ..... | 13 | 11.5 | 10.5 | 22.5 | 574.5 | 44.2 |
| 21 | 34.5 | 23 | 8.5 | ..... | 18.5 | 17 | 9 | 26 | 1,113.5 | 60.2 |
| 23 | 37 | 29 | 10.5 | ..... | 23 | 21 | 5.5 | 26.5 | 2,216.5 | 96.3 |
| 25 | 38.5 | 30 | 11 | ..... | 22 | 21.5 | 6 | 27.5 | 3,776.5 | 171.6 |
| 29 | 41.5 | 31 | 11.5 | ..... | 22.5 | 22 | 8 | 30 | 7,045.5 | 313.1 |
| Aug. 1 | 44 | 37 | 15 | 3 | 28 | 24.5 | 4.5 | 29 | 11,966.5 | 427.3 |
| 5 | 46.5 | 39 | 16 | 4 | 26.5 | 25.5 | 5 | 30.5 | 12,638 | 476.9 |
| 7 | 48.5 | 40 | 18 | 6 | 26.5 | 24.5 | 6 | 30.5 | 13,905 | 524.7 |
| 9 | 50 | 42 | 20 | 8 | 26.5 | 24.5 | 5.5 | 30 | 14,228.5 | 536.9 |
| 11 | 51.5 | 42 | 21.5 | 9.5 | 24.5 | 23.5 | 7 | 30 | 16,386 | 668.8 |
| 13 | 53.5 | 42.5 | 24.5 | 12.5 | 23.5 | 20.5 | 8.5 | 29 | 16,693 | 710.3 |
| 15 | 55.5 | 45 | 29.5 | 17.5 | 23 | 18 | 8 | 26 | 14,993 | 651.8 |

TABLE VIII.—*Actual and 2-day average increase in the number of leaf spots per plant in a medium-early and an early sugar-beet field from June 18 to September 19, at Rocky Ford, Colo., in 1913*

| Date. | Increase in medium-early field. | | Increase in early field. | | Date. | Increase in medium-early field. | | Increase in early field. | |
|---|---|---|---|---|---|---|---|---|---|
| | Actual. | 2-day average. | Actual. | 2-day average. | | Actual. | 2-day average. | Actual. | 2-day average. |
| June 20 | 0.3 | 0.3 | ..... | ..... | Aug. 5 | 203 | 102 | 3,996 | 1,998 |
| 22 | .5 | .5 | ..... | ..... | 7 | 65 | 65 | 2,167 | 2,167 |
| 24 | 0 | 0 | ..... | ..... | 9 | 127 | 127 | 2,373 | 2,373 |
| 26 | .3 | .3 | ..... | ..... | 11 | 53 | 53 | 4,208 | 4,208 |
| 28 | .1 | .1 | ..... | ..... | 13 | 190 | 190 | 1,831 | 1,831 |
| 30 | .4 | .4 | ..... | ..... | 15 | 282 | 282 | 1,450 | 1,450 |
| July 2 | 2.3 | 2.3 | ..... | ..... | 18 | 280 | 186 | 2,450 | 1,633 |
| 7 | 4.9 | 1.9 | ..... | ..... | 22 | 895 | 447 | 2,286 | 1,143 |
| 8 | 1.4 | 2.8 | ..... | ..... | 25 | 1,379 | 919 | 2,582 | 1,722 |
| 9 | ..... | ..... | 70 | 70 | 28 | 5,664 | 3,776 | 2,944 | 1,962 |
| 10 | .9 | .9 | ..... | ..... | 30 | 2,572 | 2,572 | ..... | ..... |
| 12 | .2 | .2 | 18.5 | 12.2 | Sept. 1 | 2,582 | 2,582 | ..... | ..... |
| 14 | .7 | .7 | 24.5 | 24.5 | 3 | 1,634 | 1,634 | ..... | ..... |
| 16 | .7 | .7 | 20.5 | 20.5 | 6 | 799 | 532 | ..... | ..... |
| 21 | 3.3 | 1.3 | 672 | 269 | 8 | 771 | 771 | ..... | ..... |
| 23 | 3.3 | 3.3 | 1,272 | 1,272 | 10 | 578 | 578 | ..... | ..... |
| 25 | 1.9 | 1.9 | 1,723 | 1,723 | 13 | 732 | 488 | ..... | ..... |
| 28 | 4.9 | 3.2 | ..... | ..... | 15 | 857 | 857 | ..... | ..... |
| 29 | ..... | ..... | 3,282 | 1,641 | 17 | 635 | 635 | ..... | ..... |
| 30 | 5.1 | 5.1 | ..... | ..... | 19 | 543 | 543 | ..... | ..... |
| Aug. 1 | 44 | 44 | 4,978 | 3,318 | | | | | |

The period of incubation of the fungus being from 11 to 13 days, as shown by artificial infection experiments, a corresponding increase in the number of spots on the leaves would not necessarily follow immediately after a period during which conditions favorable for infection prevailed.

Notwithstanding the early appearance of the spots in the medium-early field—on June 20—and a consequent expectation of an epidemic of the disease, the increase in infection was very light (Table VIII)

during the latter part of June and early part of July. This was doubtless due to the fact that during this period the stomata were closed against the fungus the greater part of the day on account of the excessively high temperature, which was generally above 100° F., and the excessively low humidity, which at one time fell to 10 and which was only above 60 from 6 to 15 hours a day, and also to the fact that a temperature as high as 95° inhibits the growth of the fungus and kills it after a few days.

After July 5 the temperature was lower and the humidity higher than during the period above mentioned. As a result, numerous conidia were produced from July 9 to 12, and at the end of the period of incubation—July 21 to 25—there was a slight increase in the number of spots (Table VIII). Rains between July 19 and 25 and the resulting high humidity caused a rather marked increase in the number of spots in late July and early August, these spots appearing on many leaves hitherto uninfected (Table VI). Prior to this period the number of leaves showing spots were comparatively few, but after July 30 the majority showed spots, and the proportion of infected to uninfected leaves gradually increased until August 28, after which it decreased. During the period from July 30 to August 28 the humidity was comparatively high, remaining above 60 on an average of 14.6 hours on all except three days, on which it remained above 60 for 10 hours; and the maximum and minimum temperatures generally were not above 90 nor below 55, respectively. The increased proportion of infected to uninfected leaves during this period, however, was not necessarily due to increasingly favorable climatic conditions but to the cumulative effect of the organism, the amount of viable conidia and consequent new infections increasing as the number of spots increased, as shown by the enormous increase of 3,776 spots per plant on August 27 and 28. After September 3 the increase in infection was considerably less (fig. 9). This was due apparently to the fall in temperature, the maximum being rarely above 76° F and the minimum seldom above 50° after September 8, while the humidity was comparatively favorable.

The increase in infection through the season was considerably higher in the early than in the medium-early sugar-beet field, as shown by the total amount of the disease (Tables VI and VII) and the actual increase (Table VIII and fig. 9). This was due to the fact that the foliage was heavier in the early than in the medium-early field (Tables VI and VII, functional leaves), and consequently the humidity was higher and the infection greater in the former than in the latter (Table VIII and fig. 9).

The maximum increase in spots was reached on August 11 in the early field and on August 28 in the medium-early sugar-beet field. The period of greatest increase in the disease is not its period of greatest destructiveness, however, as the plant is not immediately affected by the disease, some time being required for the leaves to be killed.

Prior to August 1, only isolated records of humidity were made in the early field,[1] but after this date continuous records of both humidity and temperatures in both fields were available for comparison (fig. 10).[2] The temperatures prevailing in the two sugar-beet fields were quite comparable, but the humidity was generally different. For instance, from August 2 to 23 the humidity remained above 60 for a longer time, and the maximum humidity was, as a rule, higher in the early than in the medium-early field; from August 23 to September 1 the maximum humidity was lower in the early than in the medium-early field; after the latter date strikingly lower, the difference ranging from 5 to 15 units; after September 5 the humidity remained above 60 for a shorter time in the former than in the latter field; but from September 6 to 21 the range of humidity in the two fields was much closer than during the periods previously mentioned.

The difference in the humidity of the two fields seemed to be due to the difference in the amount of foliage present. Early in the experiment the foliage was heavier in the early than in the medium-early field, but owing to an extremely severe infection, which developed between July 29 and August 13 (fig. 9), the relative proportion of foliage in the two fields was reversed after that period. As a result of this reversal, less moisture was retained and the humidity was lower in the early than in the medium-early field during September, and consequently at that time the relative increase in infection was less in the former than in the latter. Speaking more specifically, early in the experiment there was an average of 29 functional leaves per plant in the early field and 22 in the medium-early; on August 15 there was an average of 26 leaves per plant in both fields, while later on there were fewer per plant in the early than in the medium-early field. On the other hand, on August 13 there was an average of 23.5 infected leaves per plant, with an average of 710 spots per leaf in the early field, and on September 8 there was an average of 21.5 infected leaves per plant, with an average of 508.3 spots per leaf, in the medium-early field.

A comparison of the death rate of the leaves in the two fields before and after the disease appeared shows its destructiveness. For instance, in the early and medium-early fields, from July 7 to 29 and from July 2 to August 25, when no leaves were killed by the fungus, the death rate from normal causes was approximately one leaf per plant in three and four days, respectively; while from July 29 to August 15 and August 25 to September 19, when the disease was most severe in the two fields, the death rate averaged one leaf per plant in nine-tenths of a day and one and three-tenths days, respectively.

[1] These and the later continuous records indicate that prior to August 1 the humidity was generally higher in the early than in the medium-early field.

[2] The temperature records taken at the Weather Bureau station were included in the comparisons and were found to agree closely with those obtained in the two fields.

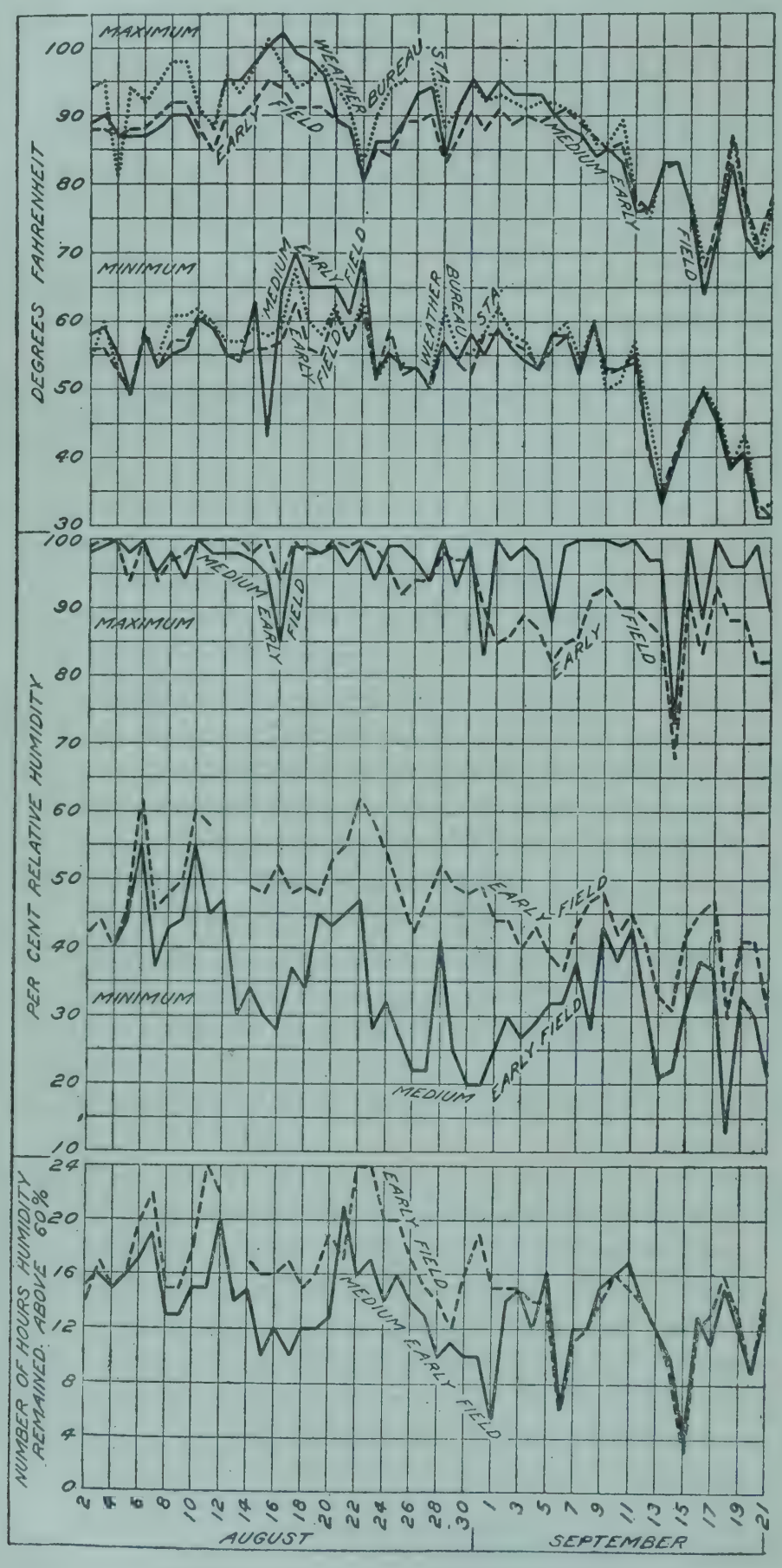


FIG. 10.—Curves of the maximum and minimum temperatures and relative humidities and the number of hours that the humidity remained above 60 from noon of the preceding to noon of the given day among the sugar-beet plants of a medium-early and an early field. For comparison, the maximum and minimum temperature records from the Weather Bureau station are included. August 2 to September 21, 1913, at Rocky Ford, Colo.

SUMMARY

(1) The life of the fungus *Cercospora beticola* overwintering in sugar-beet-top material varies with different environment. When exposed to outdoor conditions, the conidia die in from one to four months; but when kept dry live as long as eight months. The sclerotia-like bodies, which are more or less embedded in the tissues of the host, are more resistant than the conidia, living through the winter when slightly protected, as, for instance, in the interior of a pile of hayed sugar-beet tops or buried in the ground from 1 to 5 inches, and become a source of infection for the succeeding crop. Notwithstanding the difference in temperature and soil-moisture conditions, similar results from the overwintering experiments were obtained at Rocky Ford, Colo., and Madison, Wis.

(2) Climatic conditions and the development of the leafspot can be correlated only when all records are taken at the same relative positions, as shown by comparisons of the Weather Bureau records and the records taken among the plants and 5 feet above the field.

(3) The maximum temperature is much higher near the ground than 5 feet above early in the season, but the difference diminishes as the season advances.

(4) Throughout the season the maximum relative humidity was higher among the leaves than 5 feet above the field. Early in the season, while the plants were small, the humidity remained above 60 longer each day 5 feet above the field than among the plants near the ground; but after the plants attained a good size this condition was reversed. Because of this difference, only records collected among the leaves should be considered in correlating climatic conditions and conidial production and infection.

(5) The effect of rainfall and irrigation on the increase of relative humidity and its duration is apparently much the same.

(6) Thermal tests with artificial cultures showed (a) that exposure to constant temperatures of 35° and 36° C. is fatal to the growth of the fungus; (b) that growth occurred when cultures after exposure for 3 days to either of these temperatures were changed to 30.8°, and also when they were held at either for 8 hours and then at 20° for 16 hours; and (c) that a temperature of 40.5° was fatal in all combinations tested.

(7) Temperature and relative humidity influence the production of conidia and infection in much the same way. A temperature of 80° or 90° F., with a night minimum preferably not below 60°, is most favorable to conidial production, while it is checked by a temperature of 100° or higher and greatly checked by a range from below 50° to 80°. A maximum humidity ranging above 60 for not less than 15 to 18 hours each day induces a good growth of the fungus.

(8) Because of the higher humidity on the lower than on the upper surface of the leaf, the conidia are generally more abundant on the lower surface of the spots, but because of the action of rain and wind they disappear more rapidly from the upper surface.

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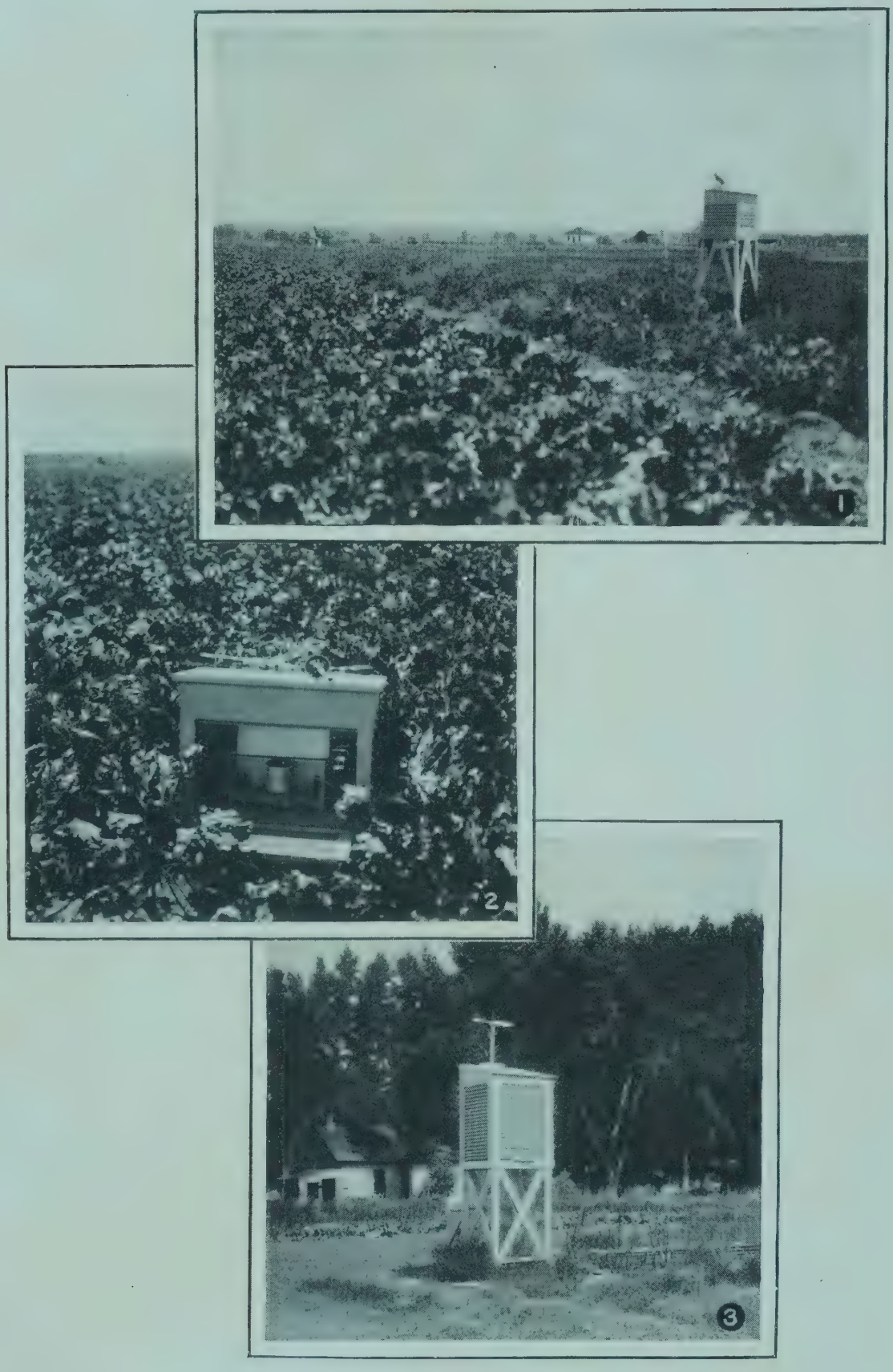
## PLATE III

*Cercospora beticola:* Overwintering tests on the experimental field at Rocky Ford, Colo., during 1912–13:

Sugar-beet leaves infected with *Cercospora beticola* (*1*) stored in soil in boxes, (*2*) buried in the ground at different depths from 1 to 8 inches, and (*3*) left exposed above the ground in a pile of hayed sugar-beet tops.

1
2
3



## PLATE IV

Field stations for the collection of weather data at Rocky Ford, Colo., in 1913:

Fig. 1.—Weather shelter, anemometer, and rain gauge at edge of sugar-beet field.

Fig. 2.—Weather shelter among beet plants, showing hygrothermograph and cog psychrometer.

Fig. 3.—Weather shelter of the local Weather Bureau station about 3 miles from sugar-beet field.

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# SOLUBLE NONPROTEIN NITROGEN OF SOIL

By R. S. POTTER, *Assistant Chief in Soil Chemistry*, and R. S. SNYDER, *Assistant in Soil Chemistry, Iowa State College Experiment Station*

## INTRODUCTION

Dilute alkali dissolves a larger proportion of the organic material of soil than any of the other relatively mild reagents. A still larger percentage is extracted from soil previously treated with 1 per cent of hydrochloric acid (HCl), and this latter reagent dissolves but little of the organic material. The term "humates" is fast disappearing from current scientific literature, yet one often reads that the reason the preliminary washing with acid renders the organic matter more soluble in the alkali is that the calcium of the calcium humates is dissolved out, making the free humic acids soluble in the alkali. To say that the proteins are rendered more soluble by the removal of the calcium and the heavy metals would explain the solubility just as well and would be more correct scientifically.

As pointed out by Lipman (4, p. 251), much of the organic nitrogen of the soil must be protein in nature. The chief sources of the nitrogen are crop residues, manures, and bacterial cells, and in these much of the nitrogen is in the form of protein. Investigations carried out in this laboratory (5) have shown that soils contain a large quantity of the so-called humin compounds. These have a great tendency to be adsorbed by such compounds as magnesium oxid and calcium hydroxid, and therefore removal of calcium from the soil by acid would tend to make these more soluble.

Upon the acidification of the alkali extract a precipitate is obtained which has been called humic acid. This term also is no longer taken seriously. It would seem that the rational explanation of this precipitate would be simply that it was made up of proteins thrown down, as salts of the precipitant, as salts of organic acids, such as nucleic acid (7), or resinous acids (6), both of the latter substances having been isolated from the acid precipitate. It would also contain, no doubt, some free organic acids.

In analyses of the solution obtained by the prolonged boiling of soils with strong acids and of the hydrolyzed humic acids by the Van Slyke method (8), it was found that the results for the humic acids did not differ markedly from the results for the organic matter of the soils as a whole from which they were derived. Since that time it has occurred to

the writers that this would hold for the material precipitated by acid from the alkali extract, but perhaps this would not be true of the organic nitrogen compounds remaining in solution. It has been pointed out by Shorey (7) that many organic compounds have been isolated from the alkali extract of soil, which, though relatively quite soluble in water, can not be detected in or isolated from the water extract of soils. Therefore it has seemed that information might be obtained relative to the degree of decomposition of the organic matter in the soil by determining the proportion of nitrogenous compounds left in solution after the precipitation of the proteins by a suitable reagent was completed. It was with these problems in mind that the preliminary investigation was carried out.

## EXPERIMENTAL METHOD

The general procedure followed was to determine the nitrogen in the alkali extract of soil with and without added material and the determination of nitrogen in the filtrate from the precipitate of the proteins in the alkali extract of soil with and without added material. The recent critical examination of a few protein precipitants by Greenwald (3) led us to use trichloracetic acid as our precipitant.

The detailed procedure was as follows: Soil, ground to pass a sieve of 100 meshes to the inch, was extracted with 1 per cent of hydrochloric acid until no calcium was found in the wash water. After air drying, 100 gm. were placed in an 800 c. c. bottle and 500 c. c. of a 1.5 per cent solution of sodium hydroxid added. After shaking the mixture for 2 hours it was centrifuged for 5 minutes in a bowl centrifuge having a speed of 18,000 revolutions per minute. Two 25 c. c. portions of the clear but deeply colored extract were analyzed for total nitrogen by the Gunning-Arnold method. Two 25 c. c. portions were neutralized with sulphuric acid, sufficient trichloracetic acid in solution added to give a 2.5 per cent solution of the acid and a total volume of exactly 30 c. c. After centrifuging, 10 c. c. portions were taken from each tube and analyzed for nitrogen by the Bock and Benedict (1) modification of the Folin and Denis method (2). This was called the soluble nonprotein nitrogen.

The same procedure was used where material was added to the soil. In the case of guanin, hypoxanthin, and glucosamin,[1] weighed portions of the compounds were added to the soil, which was then shaken with the alkali. The hydrolyzed casein was prepared as directed by Greenwald (3), which consisted, in brief, of boiling the casein for 40 hours with hydrochloric acid, the removal of the acid under diminished pressure, neutralization with sodium hydroxid (NaOH), and filtration. After mixing the filtrate with animal charcoal it was again filtered and final filtration carried out after crystallization of the tyrosin. For all the remaining materials solutions were prepared, sometimes with the aid of a little *N*/10 acid or *N*/10 alkali. Suitable amounts of the solutions were

[1] We wish to express our thanks to Prof. P. A. Kober for the samples of the hypoxanthin and guanin, and to Dr. A. W. Dox for the sample of glucosamin.

added to the soils and then sufficient alkali added to make 500 c. c. of a 1.5 per cent solution. In all cases, with the above-noted exceptions, the purest commercially available compounds were used, but analyses for nitrogen were run on the solid material when it was used, and when solutions were employed aliquots were analyzed. These determinations were also made by the micro method. It should be mentioned here that 6 minutes was found to be quite an inadequate digestion period for some of the compounds. It is believed that in some cases when apparently more than 100 per cent of the added substance was extracted from soil, faulty analysis of the substance was the cause. Insufficiency of material precluded repeating tests with many of the materials.

The soil used for all these tests was a silt loam containing 0.30 per cent of nitrogen. Samples A and B, as shown in Table I, differ only in that they were not taken from the field at the same time.

TABLE I.—*Analyses of 5-gm. portions of soil for alkali-soluble and soluble nonprotein nitrogen*

| Soil sample. | Substance added. | Nitrogen added. | Nitrogen in the alkali extract. | Nitrogen of the added substance recovered in the alkali extract. | | Soluble nonprotein nitrogen. | Soluble nonprotein nitrogen recovered. | |
|---|---|---|---|---|---|---|---|---|
| | | *Mgm.* | *Mgm.* | *Mgm.* | *Per ct.* | *Mgm.* | *Mgm.* | *Per ct.* |
| A | Nothing | ..... | 5.93 | ..... | ..... | 1.29 | ..... | ..... |
| | Hydrolyzed casein | 2.09 | 7.99 | 2.06 | 98.4 | 3.33 | 2.04 | 97.5 |
| | Amino benzoic acid | 2.14 | 8.08 | 2.15 | 100.4 | 3.41 | 2.12 | 99.0 |
| | Glutamic acid | 2.27 | 8.26 | 2.33 | 102.6 | 3.48 | 2.19 | 96.5 |
| | Hippuric acid | 2.42 | 8.40 | 2.47 | 102.0 | 3.73 | 2.44 | 100.8 |
| | Glutamic acid imid | 2.36 | 8.36 | 2.43 | 103.0 | 3.59 | 2.30 | 97.5 |
| | Succinimid | 2.53 | 8.45 | 2.52 | 99.5 | 3.86 | 2.57 | 101.5 |
| | Guanidin sulphate | 1.97 | 6.725 | 1.975 | 40.4 | 1.84 | 0.55 | 28.0 |
| | Urea | 2.48 | 8.41 | 2.48 | 100.0 | 3.66 | 2.37 | 95.6 |
| | Uric acid | 2.42 | 8.34 | 2.41 | 99.6 | 3.78 | 2.49 | 103.0 |
| | Caffein | 2.32 | 6.93 | 1.00 | 43.1 | 2.21 | .92 | 39.7 |
| | Theobromin | 1.96 | 7.90 | 1.97 | 100.5 | 3.27 | 1.98 | 101.0 |
| | Guanin | 2.49 | 8.45 | 2.52 | 101.2 | 2.325 | 1.035 | 41.6 |
| | Hypoxanthin | 2.52 | 8.34 | 2.42 | 96.0 | 3.42 | 2.13 | 84.6 |
| | Skatol | 1.26 | 7.19 | 1.26 | 100.0 | 1.80 | .51 | 41.2 |
| | Nucleic acid | 2.00 | 7.92 | 1.99 | 99.5 | 1.67 | .38 | 19.0 |
| | Cadaverin | 2.00 | 7.92 | 1.99 | 99.5 | 2.45 | 1.16 | 58.0 |
| | Amygdalin | 2.48 | 8.41 | 2.48 | 100.0 | 3.42 | 2.13 | 85.7 |
| | Peptone (Witte) | 2.98 | 8.76 | 2.83 | 94.9 | 2.12 | .83 | 33.5 |
| | Casein | 2.42 | 7.42 | 1.49 | 61.6 | 1.29 | 0 | 0 |
| | Edestin | 2.43 | 6.58 | .65 | 26.8 | 1.29 | 0 | 0 |
| | Egg albumin | 2.01 | 7.56 | 1.63 | 81.3 | 1.29 | 0 | 0 |
| | Glucosamin | 1.98 | 8.08 | 2.15 | 108.5 | 3.36 | 2.07 | 104.5 |
| B | Nothing | ..... | 6.51 | ..... | ..... | 1.25 | ..... | ..... |
| | Asparagin | 2.11 | 8.62 | 2.11 | 100.0 | 3.24 | 1.99 | 94.2 |
| | Acetanilid | 2.14 | 8.66 | 2.15 | 100.5 | 3.24 | 1.99 | 93.0 |
| | Benzamid | 2.17 | 8.69 | 2.18 | 100.5 | 3.27 | 2.02 | 93.2 |
| | Creatinin | 2.07 | 8.06 | 1.55 | 74.8 | 2.71 | 1.46 | 70.5 |

It is not thought that all the compounds used are actually present in soil. The substances were chosen rather to represent classes of compounds which conceivably might be in soils. Guanin, hypoxanthin, nucleic acid, peptone, and creatinin have been isolated from soil. It is

realized that the list is very incomplete. As opportunity to make or to purchase more compounds presents itself, the investigation will be considerably extended. From the data presented, it is observed that quite varying proportions of the pure proteins, which in reality are soluble in dilute alkali, are extracted. This seems to be a confirmation of the contention that the alkali extract as a whole does not represent a definite class of nitrogenous compounds. Of the simpler compounds, it is seen that the more acid and more closely neutral compounds are completely extracted and remain in the soluble nonprotein portion. An exception to this is found in the case of nucleic acid. This is to be expected from its tendency to combine with protein compounds to give insoluble nucleins. The action of the purin compounds is interesting. In general the more basic the compound the less the quantity recovered.

## CONCLUSIONS

(1) If the results with the pure proteins be considered, it is probable that the alkali extract as a whole contains no definite group of compounds.

(2) From the results obtained by the precipitation of the alkali extract with trichloracetic acid it would seem that the soluble nonprotein fraction may contain most of the simpler nitrogenous compounds, and therefore its determination would give an index of the degree of decomposition of the organic matter in the soil.

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# OVIPOSITION OF MEGASTIGMUS SPERMOTROPHUS IN THE SEED OF DOUGLAS FIR

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The larva of a seed chalcidid, *Megastigmus spermotrophus* Wachtl, has been very commonly recorded from the seeds of Douglas fir (*Pseudotsuga taxifolia*), but most of these records apply only to mature seed. The method by which the larvæ of this insect get into the seeds has not been previously described. The oviposition of the female, the period of the growth at which the seeds are infested, and the subsequent development of the larvæ are matters on which we have no published data.

The following is an account of the oviposition of this species observed at the Forest Insect Seed Station of the Bureau of Entomology at Ashland, Oreg., during the season of 1915.

During the season of 1914 a heavy emergence of adults of *Megastigmus spermotrophus* from Douglas fir seed of the 1913 crop occurred in the vicinity of Ashland. From stored seed kept in a rearing box the male adults began to emerge on April 12, and the females on April 16; 2,897 adults emerged from 6¾ ounces of seed, the period of maximum emergence occurring between April 23 and May 11. A number of these adults were liberated in a small cage kept in the laboratory. It was found that the adults would not live any length of time unless fed. Pieces of blotting paper saturated with sugar solution were hung in the cage and on this the adults were frequently seen feeding. Young Douglas fir cones were kept in the cage with the adults for a period of about three weeks; and although copulation was frequently observed, no attempts were noted on the part of the females to oviposit in the cones.

During the season of 1915 another effort to secure a record of oviposition in a rearing cage met with far better success. Various lots of infested Douglas fir seed were kept at the station, and the emergence of the adults from this seed was quite similar to that observed in 1914. The maximum period of emergence in the laboratory occurred between April 20 and May 2. From cones which were kept caged over winter under outdoor conditions at the same elevation, the maximum emergence occurred between May 1 and 16. At elevations of 3,000 to 4,000 feet the emergence occurred during the latter part of May, and above 4,000 feet much of the emergence occurred in June.

Many adult chalcidids were liberated at different dates between April 18 and May 20 in a cage considerably larger than that used in 1914 (Pl. V,

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fig. 1). This was kept outdoors in a partially shaded position. The adults were fed as before with sugar solution.

A Douglas fir branch bearing cones about 3 weeks old was placed in this cage on April 18. The young cones were then about 1½ inches long, the scales were still soft, and the seeds had the milky interior and unhardened coat. The base of the branch was kept in a jar full of moist earth. Fresh branches were placed in the cage at intervals until May 15, when the cones were estimated to be about half mature.

Mating was observed in this cage and in the emergence vials of the rearing boxes during the entire period. The first oviposition of a female on a cone was observed on April 20 at about 3.30 p. m. A female was observed crawling about over the bracts and feeling the scales with her antennæ. This lasted for several minutes; then the female paused on one of the exposed scales with her head pointed toward the base of the cone. After resting quietly for a moment the abdomen was lifted and at the same time the posterior end was doubled under so that the sheath of the ovipositor was brought forward between the legs until the tip rested on the surface of the cone scale at a point directly under the insect's head. The point chosen for the insertion of the ovipositor was close to the outer edge of the scale on which the female rested. The sheath of the ovipositor was then withdrawn and assumed its normal position back of the abdomen, while the ovipositor was slowly forced down into the cone. The abdomen was gradually lowered as the ovipositor was thrust into the cone until finally the entire body rested close to and in a line parallel with the surface of the cone scale (Pl. VI, fig. 3). In this position the female rested for about a minute and then withdrew the ovipositor. This was accomplished by raising the body and doubling the abdomen until it assumed a position similar to that in which the oviposition was started (Pl. VII, fig. 1). This allowed the ovipositor to be withdrawn and returned to its sheath.

The oviposition of two females was recorded on April 22 and that of the same number on April 23. Between this date and April 26 no oviposition and very little activity on the part of the seed chalcidids were observed. On the morning of April 26 a female was observed ovipositing, and this operation was recorded four times during the day. On April 27 about the same activity occurred. April 28 was a warm, sunny day and great activity on the part of the females occurred. The cage at this date contained 10 cones and about 50 females. At almost any hour during the day from one to three females could be seen either ovipositing on the cones or preparing to do so. From April 29 to May 2 cool rainy weather prevailed, and almost no activity on the part of the chalcidids occurred in the cage. May 3, 4, and 5 were warm, sunny days, and the oviposition could be witnessed at any time during the day. Oviposition in the 10 cones in the cage on these dates was in progress continuously during the day, at which time the best observations of the act were obtained.

A spell of rainy weather persisted from May 8 to 25, and no further records were secured. The subsidence of emergence after the latter date made it impossible to obtain adults for liberating in the rearing cages, and efforts to secure further records were not attempted.

Difficulty was encountered in securing photographs, as females will not oviposit if even slightly disturbed. If a cone was jarred in any way while a female was in the act, the ovipositor would be withdrawn as rapidly as possible. Even though the ovipositor was inserted deep in the cone the female would struggle to disengage it and fly away. However, by raising the glass on the front of the cage it was possible to focus a camera directly on the cones, and several pictures were obtained in this way. For the purpose of further study and dissection, a number of females were captured and killed with the ovipositor thrust into the cones. This was best accomplished by quickly immersing the cone on which the female rested in a graduate filled with chloroform. This killed the female so quickly that her efforts to withdraw the ovipositor were seldom successful. Several of the females which were killed in this position were photographed (Pl. VI, fig. 1, 2).

The time required for oviposition varies from two to five minutes. The same female was observed to oviposit five times on the same cone, and it is probable that the operation is repeated many times before the egg-laying capacity is exhausted. The point selected for the insertion of the ovipositor was always on the surface of a scale, never on a bract, and may be either on the margin or near the center of the scale. The female always assumed a position with head pointed toward the base of the cone. As Douglas fir cones were pendent at the time of oviposition, this allowed the female to stand with her head pointed upward (Pl. V, fig. 2, 3).

In cones which were dissected with the ovipositor of the female inserted it was found that the ovipositor reached the seed in a few cases only. Apparently where successful the ovipositor passes through the scale nearest the surface and underlying bracts until it reaches the second or third scale from the surface. It then follows down through the center of the last scale nearly to its base and then turns forward into the seed just ahead of it (Pl. VII, fig. 2, 3). The fact that the ovipositor was seldom found in the seed in the cones dissected is doubtless due to the fact that the female partly withdrew her ovipositor in the death struggle.

It would seem that successful oviposition occurs only when the egg is deposited in the seed, as the larvæ have never been found to work their way through the tissues of the cone, and their development is confined entirely to the interior of one seed.

Numerous cases were found in which the ovipositor did not penetrate even as far as the base of the scale. This occurred most frequently where the cones were of such an age that the scales had hardened. In these cases the tough tissues of the scales seem to bend the ovipositor out of

its course, and in a number of the dissections the ovipositor was bent and twisted around to a course directly opposite to that intended. This condition was not encountered where the cones were still young and soft. In fact, after the cones become hardened it is difficult to realize how they can become infested at all by the chalcidids.

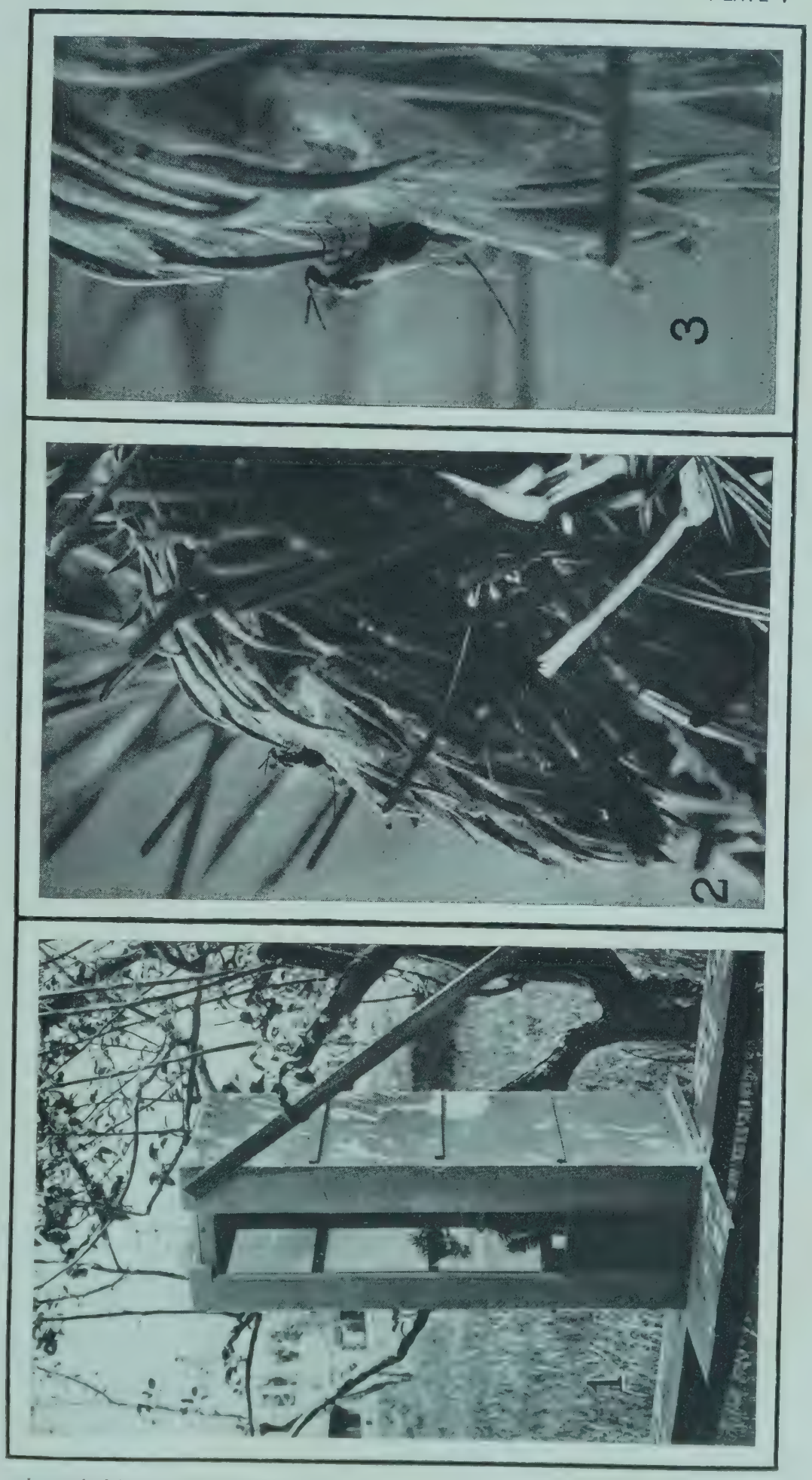
Actual oviposition in the field by the seed chalcidids was observed only once—on May 28 by Entomological Ranger J. E. Patterson. While collecting cones he noted a female on one cone with her ovipositor inserted. The insect withdrew the ovipositor and left the cone very soon after it was noticed.

## PLATE V

Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

Fig. 1.—Type of cage in which the oviposition of *Megastigmus spermotrophus* was observed. This cage was kept under outdoor conditions. A branch bearing young cones of Douglas fir was set in a jar of moist soil and kept in the cage with the females.

Fig. 2, 3.—Female resting on cone with ovipositor inserted. Photographed from life. On left, original; on right, enlargement of same to show exact attitude of the female.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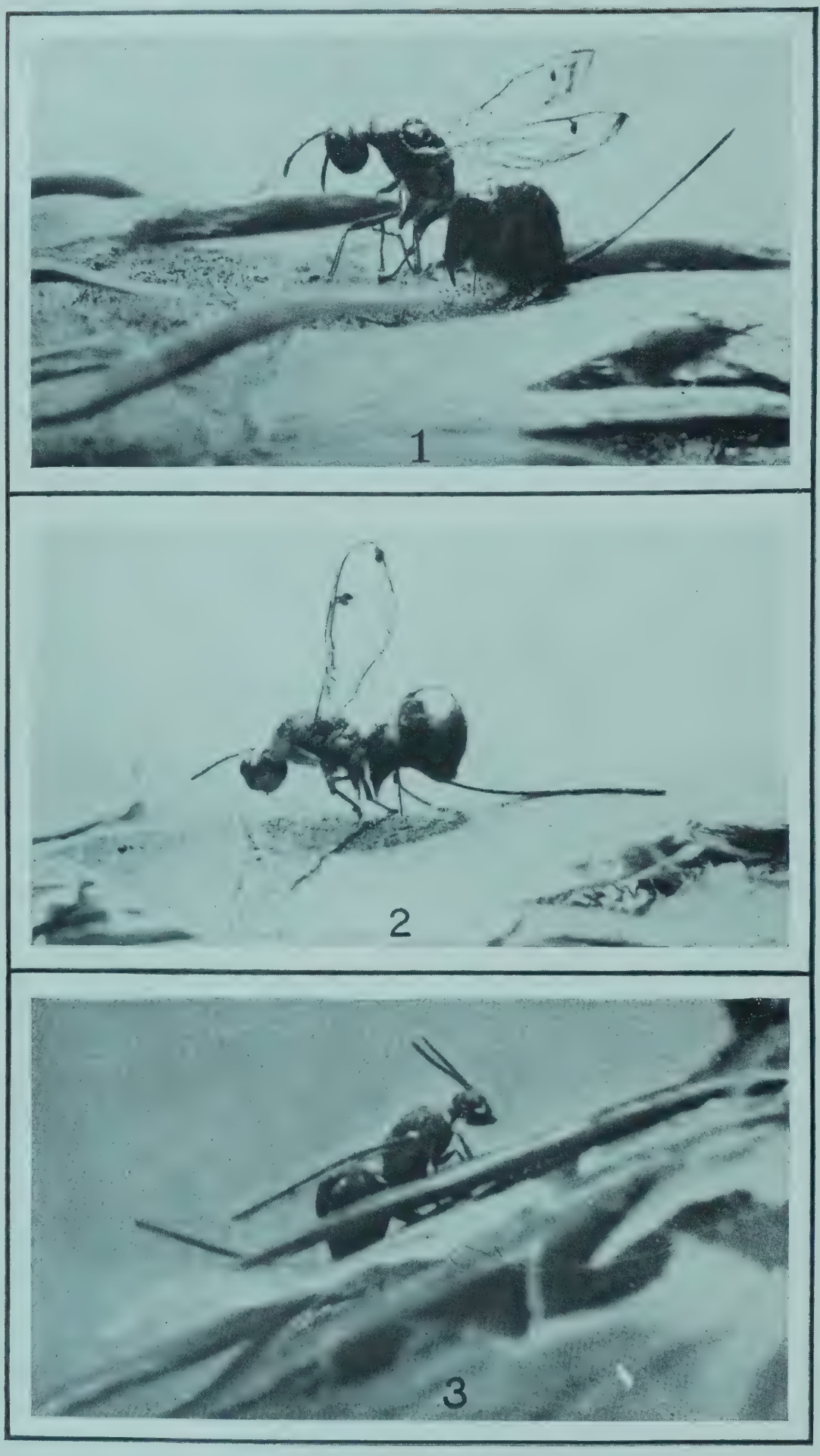
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## PLATE VI

Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

Fig. 1, 2.—Two positions of female on surface of cone with ovipositor inserted. Photographed from dead females which had been killed in this position. Enlarged.

Fig. 3.—Female resting on cone with ovipositor inserted. Photographed from life. Enlarged.

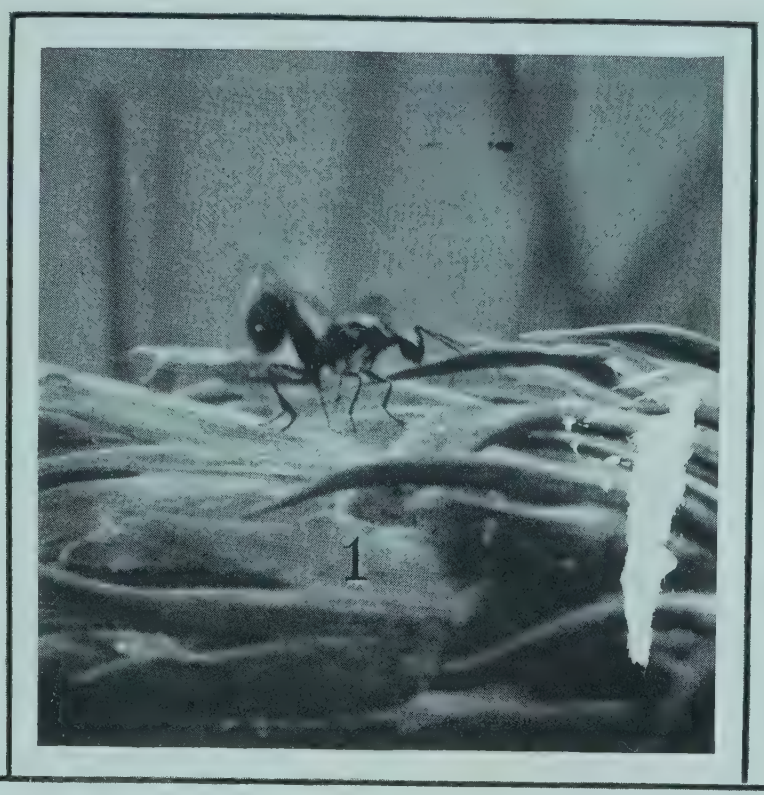
## PLATE VII

Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

Fig. 1.—Female in act of withdrawing ovipositor from cone. Photographed from life. Enlarged.

Fig. 2.—Section through a Douglas fir cone on which a female has been killed while in the act of ovipositing.

Fig. 3.—A portion of same cone and dead female with ovipositor inserted. Slightly retouched to show course followed by ovipositor in reaching the seed.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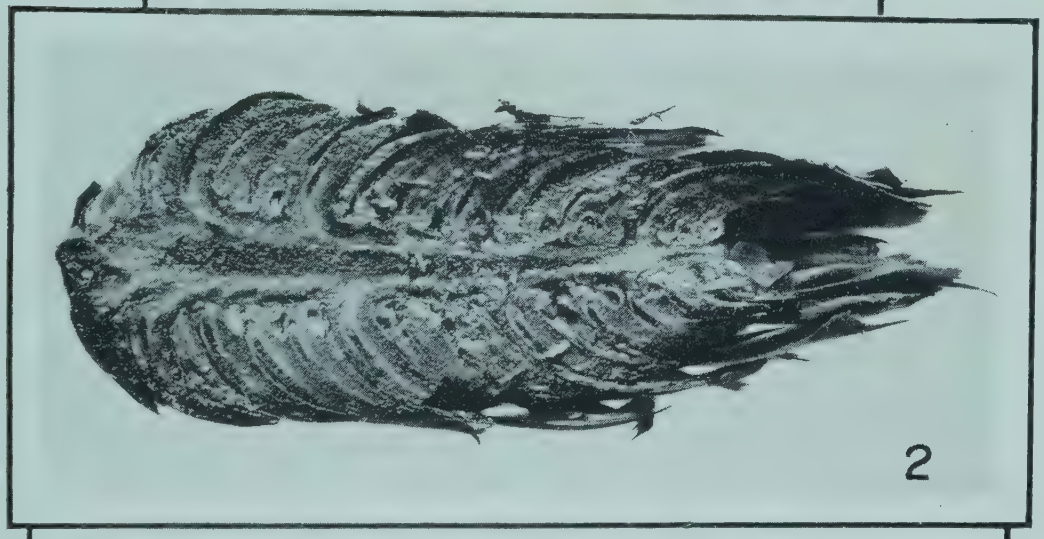
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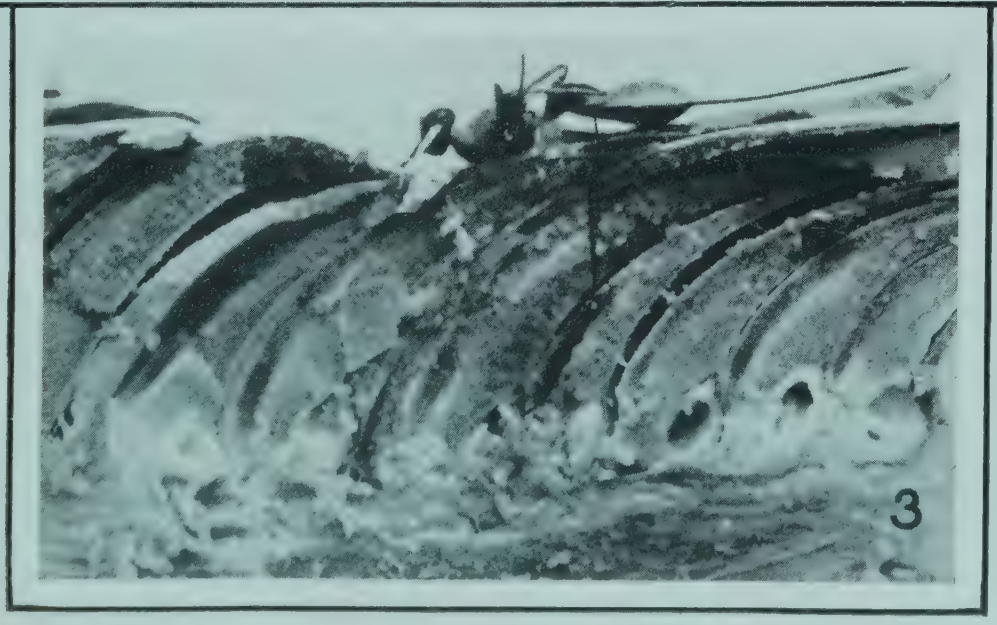
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# CITRUS CANKER[1]

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## INTRODUCTION

The ravages of certain insect pests and plant maladies have, in a considerable number of instances, been so severe as to cause intense alarm. It has been feared in the case of several crops that their culture was no longer possible in certain sections because effective means of preventing the losses resulting from such ravages were not then known. Within the last two years it has been realized that a new disease known as Citrus canker has been introduced into the Citrus-growing sections of the Gulf Coast States. This disease, beyond all doubt, is the most destructive malady affecting species of Citrus, and when it was realized that its control and eradication were so difficult, alarm concerning the future production of Citrus fruits became almost an hysteria. Those who have never seen Citrus canker under field conditions regard the reports of the highly infectious nature of this disease, of its destructiveness, and of the difficulties experienced in its eradication as the results of an overwrought imagination. The severity of Citrus canker has not been exaggerated, however, and growers should lose no time in preventing its further dissemination and in effecting its eradication.

## HOSTS OF THE ORGANISM

Citrus canker has been found to affect many of the varieties and species of Citrus, and in all probability none of the species of this genus are entirely immune. It is perhaps productive of more serious injury to the varieties of grapefruit, or pomelo (*Citrus decumana*), than to any other of the Citrus fruits. Seedling grapefruits appear to be more susceptible to canker than the budded varieties. Some regard the injury to the hardy or trifoliate orange (*Citrus trifoliata*), which is extensively used as the stock upon which to bud other species of Citrus, as equally severe. Certain of the varieties of round oranges (*Citrus aurantium*) are known to be very susceptible to Citrus canker and under favorable conditions suffer as severe injury as grapefruits. The disease occurs also on varieties of the sweet orange. Oranges of the mandarin group (*Citrus nobilis*),

[1] Published with the permission of the Director of the Alabama Experiment Station.

[2] The writer is greatly indebted to his colleague, Dr. J. S. Caldwell, for suggestions and material aid during the progress of this investigation and for assistance in the preparation of the manuscript. Much of the chemical portion of the investigation would have been impossible but for the skillful and arduous assistance of Messrs. A. C. Foster and C. W. Culpepper, formerly laboratory aids in the Department of Botany of the Alabama Polytechnic Institute. To each of these gentlemen grateful appreciation for the several services is hereby acknowledged.

including mandarins, tangerines, and Satsumas, have also been found to be diseased. The disease has been observed, too, on several varieties of lemons (*Citrus medica*) and limes (*Citrus limetta*). Thus far Citrus canker in Alabama has not been found to attack kumquats, the four species of which Swingle (17) [1] regards as belonging to the genus Fortunella. It has been observed, however, on the leaves and twigs of the kumquat in Louisiana. Swingle (18) reports its occurrence on this host in Japan.

## HISTORY OF THE DISEASE

Citrus canker is not of American origin, but beyond doubt was introduced into the Gulf States from Japan. This statement is supported by the fact that it is known to occur in Japan and the Philippine Islands (18), and, so far as can be learned, it appeared in the United States several years ago simultaneously with the importation of Satsuma and trifoliate stock into Texas in order to supply the large demand for trees for Citrus plantings. Whether it is indigenous to Japan is not known, but it is probably native of parts of eastern Asia. Since its introduction into Texas it has been disseminated by the shipment of diseased trees to other States and has further been introduced by shipments to these States direct from the Orient, so that it now occurs in parts of Florida, Alabama, Mississippi, Louisiana, and Texas.

Citrus canker had probably been present in the United States for five or six years before it was recognized as a new Citrus disease. Specimens were first collected in September, 1912, but it was not until July of the following year (1) that the Office of Nursery Inspection of Florida realized that these specimens did not represent an unusual manifestation of scab caused by *Cladosporium citri*. This mistake in diagnosis had also been made by inspectors in other Gulf States and by officers of State Experiment Stations and of the Federal Department of Agriculture. Japanese authorities had also mistaken this disease, since specimens received at the Florida Agricultural Experiment Station (2) from Japan had been identified as scab. The disease was brought to the writer's attention in February, 1914, and has been interruptedly studied by him since that time.[2]

A number of publications upon Citrus canker, all preliminary in nature, have appeared. These papers call attention to the presence of the disease in the several States, briefly describe its appearance, and recommend concerted cooperation in its eradication. The disease was first regarded as of fungoid origin, and the first claim that bacteria are the primary cause of the disease was made by Hasse (6). The present publi-

[1] Reference is made by number to "Literature cited," pp. 98–99.

[2] The writer severed his connection with the Alabama Agricultural Experiment Station on January 1, 1916. This study therefore is incomplete, time not having been afforded for verification of all portions of the study, and certain problems which have appeared in connection with the work have not been investigated. However, it was deemed advisable to record the results of the studies thus far conducted.

cation has for its purpose the recording of studies which are in part confirmatory of previous studies (2, 5, 6, 16, 19) and which further contribute to our knowledge of this disease.

## ECONOMIC IMPORTANCE

The serious nature and unusual virulence of Citrus canker and the jeopardy in which it has placed the Citrus industry can best be realized when it is recalled that the Federal Horticultural Board, on January 1, 1915, placed a quarantine on the importation from all foreign countries of Citrus nursery stock, including buds, scions, and seeds, in order to prevent further introduction of the disease into the United States. It is difficult to obtain figures as to the number of nursery and orchard trees which have been destroyed in an effort to eradicate the disease from the Gulf coast. It is equally difficult to obtain accurate figures on the amount of money which has already been expended by the Federal Government, together with the State horticultural boards, liberally aided by various organizations and by private subscriptions, in an effort to stamp out Citrus canker. Suffice it to say that the actual cost in money for eradication and for trees destroyed has been enormous.

## SYMPTOMS OF THE DISEASE

Citrus canker affects the leaves, twigs, larger branches, and fruits in a characteristic manner. Upon any of these parts the diseased areas are light brown in color and project more or less above the surrounding tissues. The cankerous areas consist of a corky mass of cells covered by a lacerated grayish membrane. It can be determined with certainty without a microscopic examination in case one has typical diseased material, and in case one has seen the disease in the various stages of development under field conditions. It is sometimes impossible to be certain whether meager specimens such as are sometimes sent in for identification are affected with canker or with some other leaf trouble. This is especially true in the case of canker on the Satsuma orange. If, however, one is permitted to make a field examination, and can thus learn of the origin of the trees, and can also observe adjacent trees, typical material may be found if Citrus canker is present.

### OCCURRENCE ON THE LEAVES

The first evidence of canker on the leaves is the appearance of very small oily or watery dots on the lower leaf surface. They may appear on either surface, but are more commonly found on the lower leaf surface. They are of a darker green color than the surrounding leaf tissue and may at this stage be mistaken for oil glands (fig. 1, 2). The diseased areas are slightly convex, however, and within a few days will have extended through the leaf, appearing on the upper surface as

greenish yellow spots. By continued development the convex surface of the spots comes to be more and more elevated until the epidermis is broken by the increased tension and the subjacent tissues are thus exposed to desiccation. The exposed tissues then become corky, darkening with age. The ruptured epidermis is turned back irregularly and persists as a lacerated membrane. The margin of the diseased area maintains an oily appearance even after the spots have ceased to increase in size. Mature spots (Pl. XI, fig. 1) vary in size from very minute to a quarter of an inch in diameter and are typically circular in outline. They may occur singly; or when they are very numerous, fuse, thus forming large, irregular areas. Cankered areas are typically elevated on both leaf surfaces. In the case of canker on Satsumas (Pl. VIII, fig. 2, and Pl. IX, fig. 4), however, there is little or no elevation of the upper leaf surface. Neither is the oily margin so evident on this host, especially in case of old cankers, in which diseased tissues have become dark brown, simulating the appearance of melanose. The appearance of the disease on leaves of *Citrus trifoliata* as shown in Plate X, figure 1, is very similar to that on grapefruit. Stevens (2) reports that he has never found Citrus canker on trifoliate orange leaves. The uninvaded tissues surrounding the cankers are paler green than the normal tissue and gradually form a chlorotic or yellowish zone (Pl. VIII, fig. 1, and Pl. X, fig. 6), which may invade all the tissues not actually occupied by the cankers. At this stage considerable defoliation, especially in the case of grapefruit and trifoliate oranges, may occur. Cankers on the leaf petioles cause defoliation even though the leaves are otherwise uninvaded.

FIG. 1.—Diagrammatic representation of young open type of Citrus canker of half the diameter of the one shown in figure 2. *pp*, Palisade parenchyma; *ue*, upper epidermis; *le*, lower epidermis; *d*, diseased tissues: *a*, air space arising from tensions due to the enlargement of cells and disintegration of tissues.

### OCCURRENCE ON THE TWIGS AND BRANCHES

Limb canker appears more commonly on very young twigs because of the absence of any considerable suberization, but larger branches are subject to infection. Growing cankers have been observed on limbs ½ to ¾ inch in diameter (Pl. VIII, fig. 3, 4). The disease has been found on branches of grapefruit, trifoliate oranges, lemons, Satsumas, and certain varieties of round oranges. Cankers on twigs are first apparent as small, circular, watery spots. They rapidly enlarge, become blister-like and the epidermis ruptures, exposing the cankerous tissue below. At this stage they project more or less prominently and are very similar

in appearance to the spots on the foliage. Isolated cankers remain circular in outline. When the spots originate close together, however, large irregular, variously cracked or fissured cankers are developed, which may involve an area several inches in length. The epidermis persists as a grayish broken membrane at the margin of these cankers (Pl. VIII, fig. 5). Twigs and larger branches may be completely girdled, resulting in the death of the distal parts. Affected trees exhibit a stunted growth, and numerous branches may be developed below the dying tips.

The disease is very severe upon stems of grapefruit and trifoliate oranges. On the latter host the thorns are abundantly cankered and the base of the thorns appears commonly to be the initial seat of infection.

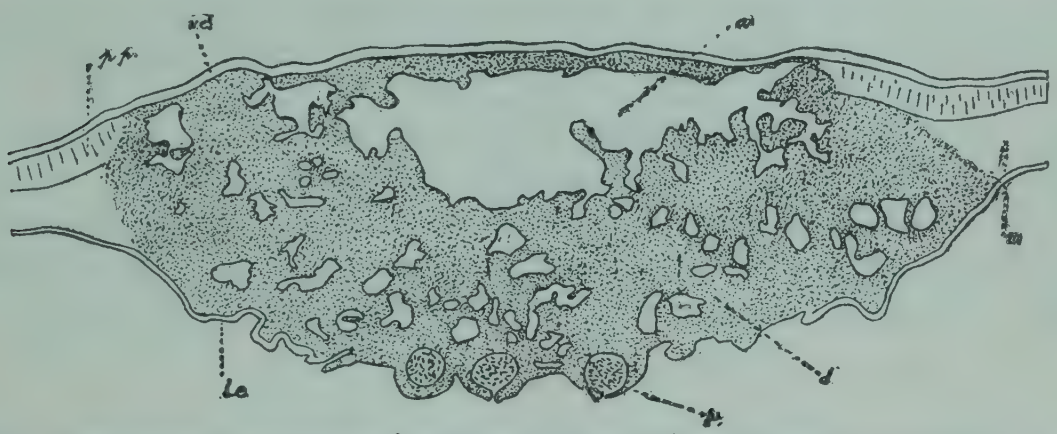

FIG. 2.—Diagrammatic representation of canker on old Citrus leaf: *pp*, Palisade parenchyma; *ue*, upper epidermis; *le*, lower epidermis; *p*, pycnidium of *Phoma socia*; *d*, diseased tissues; *a*, air space arising from tensions due to the enlargement of cells and disintegration of tissues.

Limb cankers on trifoliate oranges oftentimes are zonate with different shades of brown, especially if the outer membranes have not yet been ruptured.

## OCCURRENCE ON THE FRUIT

The cankerous areas on the fruits are quite similar in appearance to the leaf cankers, differing mainly in the larger size of the former. They are scurfy elevations, for the most part circular in outline and surrounded by a zone of chlorotic rind tissues. The corky diseased tissues are quite superficial; and if the spots unite, large scaly areas are formed (Pl. X, fig. 2). In this case the fruits may crack open because of their increase in size owing to the growth of the fruits and may become prematurely yellow and drop. Fruits which are badly cankered and have burst open are, of course, subject to invasion by various organisms of decay. Even if they remain on the tree, they are rendered very unsightly and are unsalable.

## OCCURRENCE ON THE BUDS

Nurserymen experience considerable losses from failure of Citrus buds to unite with the stock. In some cases when *Citrus trifoliata* seedlings affected with canker are used as stock, losses of over 50 per cent have

been sustained. The operation of budding either directly conveys the organisms into the wounded tissues or they are subsequently washed into them from cankers above the insertion of the bud before union has been effected.

## ETIOLOGY OF THE DISEASE

The primary cause of Citrus canker is a bacterial parasite, *Pseudomonas citri* Hasse (6). Hasse isolated this organism from cankers on grapefruit and proved it to be pathogenic to grapefruit seedlings. This claim was established at a time when the disease was regarded as of fungus origin. Hasse further pointed out the fact that a number of fungi were isolated from old Citrus cankers. The writer had found a fungus, as had also Prof. H. E. Stevens, of the Florida Agricultural Experiment Station, belonging to the form genus Phoma, commonly associated with cankerous tissues. The writer's initial inoculations were made not with pure cultures of Phoma, as has subsequently been learned, but with cultures which had overrun the bacterial parasite. Successful infections reported in the previous publication (19) are thus accounted for. Consideration will be given in another part of the present report to the part which *Phoma* spp. and certain other fungi play in the production of Citrus canker.

## PATHOGENICITY

*Pseudomonas citri* has repeatedly been isolated during the past season from cankers on grapefruit, trifoliate orange, lemon, and Satsuma oranges. The strains from these different hosts present the same cultural characters. Because of this, together with the added fact that no difficulty has been experienced in making cross inoculations, the strains are regarded as identical.

The plants used in making the inoculation experiments were grown in the greenhouse at Auburn, Ala. Typical cankers have been produced on McCarty and seedling grapefruits (Pl. IX, figs. 1, 2), pineapple oranges, Satsuma oranges, and seedling trifoliate oranges. Infections on all these species were as readily secured, whether the organism had been isolated from *Citrus trifoliata*, Satsuma, grapefruit, or lemon. Neither was there any evident difference in virulence of any of the strains. A suspension of the organism taken from pure cultures grown either on potato cylinders or in bouillon was used in making the inoculations. This suspension when applied with an atomizer resulted in a high percentage of successful inoculations. A greater number of successful inoculations were secured, as would be expected, when the plants were covered with bell jars to prevent the too rapid evaporation of the moisture. When the inoculum was introduced into the tissues of leaves, stems, or fruits through needle punctures, cankers developed in all cases. In some cases the suspension was applied to leaves with the fingers. They were dipped into the suspension

and the material was then applied by gently rubbing the leaves between the thumb and fingers. In a few cases it was arranged so that twigs bearing young leaves could be immersed for an hour or two in a bacterial suspension. Leaves inoculated in this manner are shown in Plate VIII, figure 2. It is to be noted that the infections are so numerous as to involve the greater part of the lower leaf surface.

The period of incubation appears to vary, depending on temperature, moisture, and age of the plant tissues. Very definite signs of the disease have been noted within 72 hours after inoculation. In other cases 10 days were required before the infections were evident to the eye. The longest periods were secured on Satsumas.

An organism of the same color as *Pseudomonas citri* and similar in appearance on certain media, but which does not exhibit the characteristic growth of *P. citri* on potato cylinders, has commonly been isolated from old cankers. This organism has not been found to be pathogenic on species of Citrus, however. There can be little doubt of the pathogenicity of the organism concerning which Hasse made her preliminary report (6). It is to be noted that her Plate X, figures *A*, *B*, represent natural infections and Plate X, figure *C*, artificially produced cankers. These, however, are regarded as identical in appearance. Artificially inoculated seedlings are represented also in Plate IX. As can readily be seen, the artificial cankers are much more prominently projecting than natural ones, are evidently greenish white in color, and there has been no discoloration of the leaf tissue surrounding the spot. The writer has never, under field conditions, seen specimens which resembled these artificial inoculations represented in Hasse's Plates IX and X, and he, furthermore, has examined fresh specimens in various stages of development sent from Florida, Alabama, Mississippi, Louisiana, and Texas. However, cankers similar in appearance to Hasse's artificial cankers have been produced in the greenhouse. Following her suggestion that the open, spongy type of canker is due to favorable conditions of moisture and temperature, seedling grapefruit which had been atomized with a suspension of *P. citri* were kept continuously covered with a bell jar. They were watered sufficiently often so that the air under the bell jar was maintained at a high relative humidity. Within 10 days the cankers shown in Plate IX, figure 3, had developed. These are regarded as similar in appearance to those previously produced by Hasse and represented in her Plates IX and X.

## DESCRIPTION OF PSEUDOMONAS CITRI

The primary cause of Citrus canker is a yellow, 1-flagellate organism. Its motility can be observed when taken directly from young cankers and examined in a drop of water. In this case it will be found to occur singly or in pairs. On solid media it may form into chains of six or more elements. It is quite variable in shape and size. When taken

from young cankerous tissues it is usually a short rod with rounded ends which measures from 1.5 to 2.5 by 0.5 to 0.75$\mu$. In old cultures the elements may be ellipsoidal. No endospores have been demonstrated; nor have involution forms been observed.

The organism stains readily with solutions of carbol fuchsin, analine gentian violet, and methylene blue. Only negative results have been secured with Gram's stain. When the organism has been grown on potato cylinders and is stained with anilin gentian violet, it has an apparent capsular portion (fig. 3). This capsular portion gives rise, no doubt, to the viscidity which characterizes its growth on steamed potatoes. The slime on old potato cultures can be drawn out an inch or two and does not dissolve readily in liquid cultures.

Young cultures of this organism on steamed potato cylinders have a very characteristic appearance. The growth is bright yellow, smooth, moist, glistening, and raised, with a narrow white zone along the margin of the bacterial growth. This white margin does not persist, since by its rapid growth the organism covers the entire surface of the medium. It acts very strongly on potato starch, as indicated by the entire absence of an iodin reaction on steamed potato cylinders 6 to 8 weeks old. The middle lamellæ in such old cultures have been dissolved, and the empty cells can readily be separated from one another.

FIG. 3.—*Pseudomonas citri*: *a*, Stained with carbol fuchsin; *b*, stained with Williams's flagellar stain (adapted from Hasse); *c*, stained with anilin gentian violet.

The organism has been grown on nutrient agar made by adding a water extract of corn meal, bean meal, green beans, cowpeas, potatoes, rice, orange juice, or orange leaves and stems, but the growth on none of these media is characteristic. No attempt was made to titrate any of these media to determine their acidity or alkalinity.

Colonies appear on the second day in poured plates of green-bean agar kept at room temperature. Within four or five days the surface colonies in poured plates will have become 2 or 3 mm. in diameter. The margin of the colonies is entire, and they are opaque yellow in color. They are appreciably raised and have a smooth, wet-shining surface. The character of the margin and of the surface is shown in Plate XI, figure 4. It will be noted that the reflection of the two windows in the room in which the exposure was made is shown in each of the colonies.

A filiform growth, following the line of the stroke and widening at the base of the slant, is formed in stroke cultures on green-bean agar. The growth does not penetrate the agar and does not give rise to the production of any stain or odor. In stab cultures on this medium a filiform but otherwise nontypical growth is produced, which when viewed from

above appears like the surface colonies in poured plates. Growth in stab cultures on various media is always or nearly always best at the surface of the media.

On nutrient-gelatin plates the colonies are circular in outline, slightly raised, entire margined, and yellowish. In gelatin stabs a filiform growth appears along the line of puncture, with the greatest growth at the surface of the medium, and with a rather slow liquefaction.

The organism is regarded as a facultative anaerobe. No gas is formed in fermentation tubes containing a 2 per cent solution of Witte's peptone. With this as a basal solution, five solutions were made by adding 1 per cent of one of the following carbon compounds: Saccharose, dextrose, lactose, maltose, and glycerin. All inoculated tubes developed a slight cloudiness, which extended into the closed end of the tube by the second day. More vigorous growth occurs in the open end of the tube, however, and after four or five days the cloudiness is very marked. Yellowish flocculent particles appear later in the open end, and a yellowish ring is formed at the surface. No gas formed in any of the solid media in which the above-mentioned carbon compounds were added to the nutrient agar.

In stab cultures on litmus-dextrose, litmus-lactose, litmus-saccharose, and litmus-glycerin agar no gas formation was apparent in 10-day old cultures. It is not known whether acidification will occur in old cultures on these media.

In sterile tubes of litmus milk there is a rather slow reduction of the litmus. After five days there is a slight increase in the blue color. The reddish whey is gradually formed on the surface, and the casein is precipitated.

There is no reduction of nitrates in Witte's peptone solution containing a trace of potassium nitrate. Phenoldisulphonic acid was used as a reagent 10 days after the date of inoculation, at which time both the check and the solution in which the organism was growing were colorimetrically alike.

Only negative tests for indol were secured in peptonized beef-bouillon cultures. A very conspicuous clouding occurs in this medium within 24 hours after inoculation. As these cultures get older they become somewhat flocculent, and a yellowish ring is formed at the surface of the media.

The thermal death point, as found in preliminary tests, was between 58° and 70° C. In order to determine more nearly the point, tests were made by exposing the organism taken from potato cylinder cultures, and transferred to tubes of bouillon. The tubes were then placed in a water bath for 10 minutes at some given temperature between these limits. The temperature of the bath was kept constant during the period of exposure. The tubes were subjected to room temperature for several

days to observe the development of cloudiness. In order to be certain, however, of the viability of the organism, loops of bouillon from these tubes were transferred to planted plates of nutrient agar, and the subsequent development noted. No growth occurred in the tubes exposed at temperatures above 65° C.

No attempts have been made to determine the exact degree of tolerance of this organism to acids. When transfers were made to dextrose-peptone agar +10, +20, and +40 Fuller's scale, it was found at the end of three days to have grown in the first two, but growth was completely inhibited in +40 acid. Hydrochloric and citric acids were employed in acidification.

The organism seems to exhibit a very considerable resistance to drying. In the desiccation experiments bacteria from vigorous pure cultures on potato plugs were smeared by means of a sterile platinum needle on clean miscroscopic slides in moist chambers. The moist chambers containing the microscopic slides were sterilized prior to transferring the bacterial smear to the slides. These preparations were made on June 1, and placed in a wall closet in the laboratory. On July 1, August 1, and September 1 several of the microscopic slides were removed from the moist chambers and placed in sterilized Petri dishes, using proper aseptic precautions in making the transfers. Tubes of melted nutrient agar which had been cooled almost to the point of solidification were poured upon these smeared slides. No growth occurred in the case of those tested on September 1, but those tested on July 1 and August 1 were still alive. From this it is believed that the organism can retain its viability for about two months.

The group number according to the descriptive chart of the Society of American Bacteriologists is 221.3332513.

## LIFE HISTORY OF THE ORGANISM

*Pseudomonas citri*, so far as is known, passes its entire life cycle under natural conditions within the tissues of the host. New infections appear in spring shortly after the new growth has begun. In southern Alabama the first appearance of Citrus canker in the field was noted on May 11, in 1914, and on May 27, in 1915. Old diseased areas on the foliage together with the cankers on the twigs and larger limbs are undoubtedly the source of infection in the spring. New leaves formed near old twig cankers are especially liable to become diseased first. Infections are not confined to the new growth, however. Old diseased areas on leaves and branches may enlarge by the renewed growth of the organism which has remained dormant on the margin of the old cankers. New cankers may also develop on old foliage and twigs, especially near the old, actively growing cankers. Under favorable conditions new infections may appear at any time throughout the growing season of the host. In one instance

new infections are known to have appeared abundantly under field conditions during November, 1914. Old leaves on the ground may possibly harbor the organism and there it may remain viable for a long time. Unsuccessful attempts, however, have been made to recover the organism from leaves kept in the laboratory from September, 1914, to May, 1915; nor has recovery been possible in the case of twig cankers kept under laboratory conditions from March to October, 1915.

It is believed, moreover, that the organism survives the winter in fallen leaves and that these fallen leaves constitute a very important source of infection in the following spring, especially in the case of nursery trees which have been planted between diseased grove trees.

There is every reason to believe also that the organism can remain alive in soil. This is evidenced by numerous instances in which new sprouts have come up from the roots of diseased trees which had been burned. A large percentage of these sprouts are early found to be diseased. Furthermore, the leaves on the lowermost branches or those in actual contact with the soil are commonly the first to become diseased.

The fact that the stomata, or breathing pores, on species of Citrus occur only on the lower leaf surfaces and that infections developed only on the lower surface of the leaves in all of the inoculation experiments in which the plants had been sprayed with bacterial suspensions led to the inference that the canker organism must gain entrance to the leaves through the stomata. That such is the case was established by leaf sections which were fixed 72 hours after inoculation and which were subsequently properly infiltrated, cut, and stained (fig. 4). Lenticels very probably serve as portals of entrance for the organism into the stems. A film of moisture on the surface of the leaf, twig, or fruit enables the organism to move about and thus to gain entrance into the substomatal cavity. Under ordinary conditions inoculation will be successful only in the presence of moisture. Wounds or abrasions from any cause may afford an entrance to the bacteria. Inoculations not infrequently occur through wounds made by thorns. Inoculations on leaves made by thorn scratches are shown on Plate X figure 3. Thorns which come in contact with limbs near by may inflict wounds which have subsequently been observed to be the point of origin of limb cankers. Cankers have also been found at the point of contact of limbs which rub together through movement by the wind.

When once the bacteria have passed through the stomata into the substomatal cavities, they multiply rapidly and effect a passage between the host cells to the intercellular spaces which become filled with solid masses of bacteria. As the bacteria continue to multiply, the cells farther away from the substomatal chambers become involved seriatim. In this way an area circular in outline and extending entirely through the leaf comes to be invaded. Various stages of invasion of the leaf tissues have

been observed in serial paraffin sections. Within three to five days after inoculation the disease is evident in the form of oily or watery spots. Within another week with favorable weather conditions and on young leaves the epidermis will have ruptured on one or both surfaces and open cankers will have formed. At this stage, before the exposed cells have become desiccated, the greatest danger of spreading the infection exists. Young tender tissues seem to be more susceptible to infection at this time than mature tissues. The disease progresses more rapidly, too, in young tissues than in older parts.

FIG. 4.—Early stage of Citrus canker in cross section on a young leaf of seedling grapefruit. The leaf was inoculated by immersion in a suspension of *Pseudomonas citri* from pure culture. The material was collected 72 hours after inoculation. It was then killed in strong alcohol, embedded in paraffin, sectioned, and stained with carbol fuchsin. The organism entered the leaf through the stoma, multiplied in the substomatal chamber, and spread to adjacent intercellular spaces. Drawing made with a camera lucida. × 600.

## RELATION BETWEEN PARASITE AND HOST

No study has been made other than the preliminary account of Hasse (6) of the effects of *Pseudomonas citri* on Citrus tissues. She states (p. 98) that—

> There is a rapid development of cells, and the tension resulting from the abnormal growth quickly ruptures the epidermis. The cells are found to be filled with short rod bacteria. All the cells exhibit more or less enlargement. In later stages in the development of the canker some of the cells disintegrate, and lesions are formed. The organism appears to act more vigorously on the cell contents than on the cell walls, and in due time the cell contents are exhausted. The cell walls which remain become suberized.

This problem was first attacked by making a histological study of the diseased tissues. For this purpose cankers in various stages of development on fruits and leaves were cut out so as to include some of the surrounding healthy tissue. Cankers which had developed under conditions of very high relative humidity (Pl. IX, fig. 3, and Pl. X, fig. 4) and which were consequently of the spongy type and white in color yielded especially interesting results. This white color is due to the presence of air between the cells and can be made to disappear if the cankers are immersed in water. These excised cankers were then killed in strong alcohol, embedded in paraffin, sectioned, and stained with carbol fuchsin. This stain renders the bacteria bright red, making it easily possible to determine their position within the tissues.

Contrary to Hasse's observation, the bacteria teem around and between the host cells, being present in especially large numbers in the intercellular spaces (fig. 5). When the organism occurs within the

cells, one is led to conclude, since they appear to be confined to such cells, that entrance was effected after some mechanical rupture of the host cells.

A microscopic examination of sections of young spongy cankers in which there has been no desiccation from contact with the air shows that the host cells are not killed at first. Instead, they are considerably hypertrophied and become lightly attached to each other, as shown in figures 6 and 7. In fact, if fresh cankers are cut off with a sharp razor and mounted on a slide in a drop of water, some of the host cells separate intact and of their own accord from the mass of cankerous tissue. Little if any hyperplasia is believed to occur. It is highly improbable that cell division would occur in cells in which such profound changes were taking place. It is evident from figures 6 and 7 that the enlargement of cells already present would account for the production of the cankerous tissues. The same is believed to be true in Plate X, figure 1, illustrating Hasse's observations. It is not clear, however, from her explanatory statement that "there is a rapid development of cells" whether hypertrophy or hyperplasia is meant. Death of cells in the later stages of development of canker is probably caused by drying (Pl. IX, fig. 5). The dried cankerous tissues gradually become suberized.

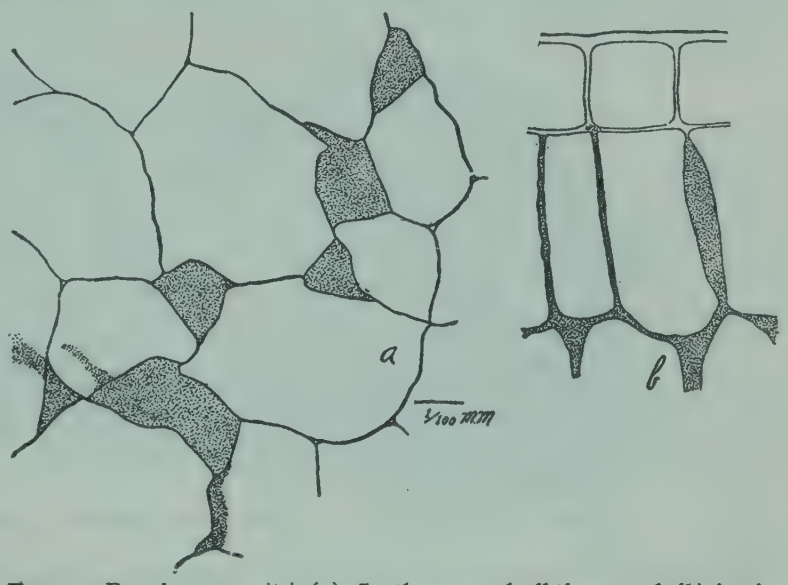


FIG. 5.—*Pseudomonas citri:* (*a*), In the mesophyll tissue and (*b*) in the palisade parenchyma. This material was fixed in strong alcohol, infiltrated with paraffin, sectioned, and stained with carbol fuchsin. Outlined with a camera lucida.

To explain the enlargement of the cells and their separation from each other, two hypotheses are advanced: First, the middle lamellæ are dissolved by an enzym, pectinase, secreted by the bacteria; second, osmotic pressure of the colloidal cell contents is modified so that the cells have a greater affinity for water. Evidence in support of both hypotheses has been secured which in part, at least, explains these interesting phenomena.

An attempt was made to demonstrate the secretion of pectinase by *Pseudomonas citri* by the following method: Six flasks of bouillon were inoculated with pure cultures of the organism. It was realized that the production of enzyms is largely dependent on the nature of the culture medium and that pectinase might be formed only within the host tissues. For this reason grapefruit leaves were placed in three of these flasks of bouillon prior to their sterilization and inoculation. After the organism

had grown in the flasks for four weeks, the bouillon was filtered through a Chamberland filter. This filtrate contained no living organisms, as demonstrated by transfers of platinum loopfuls to agar plates, with no growth on these plates after three days. When at the end of three days it was known that the filtrate was sterile, fresh grapefruit leaves were introduced into the filtrate. These leaves were sterilized, prior to their introduction, by immersion for half a minute in 1 to 1,000 bichlorid of mercury and by rinsing them subsequently in three changes of boiled tap water. Negative evidence of the presence of living organisms in the filtrate containing the grapefruit leaves was secured by agar plates made one week after the introduction of the leaves into the filtrate. An examination of the leaf tissues at the end of two weeks showed no evidence of dissolution of the middle lamellæ. This was true in the case of the filtrate obtained from both sets of the six original flasks.

In another experiment Irish potatoes were cut into slices and placed in moist chambers on moist filter paper. *Pseudomonas citri* was then

FIG. 6.—Drawing of a stained section of a natural canker on grapefruit.

transferred to these cut surfaces. Within a week hemispherical areas in which the cells were easily separable one from the other had been formed immediately beneath the colonies. That *P. citri* alone had caused this condition was shown by the reisolation in pure culture of this organism from the softened potato tissues. Because of this result, together with the fact, previously indicated, that the cells of cankerous tissues are so easily separable, and in spite of the negative evidence of enzym secretion in bouillon culture, it is believed that pectinase is secreted by the parasite.

The fact of the increased size of cells of cankerous tissue in itself supports the hypothesis that there has been an increased osmotic pressure within affected cells. Several facts contribute toward solving the question of how this increased pressure is brought about. In the first place the cell contents must manifestly be modified by the dissolution of the middle lamellæ, since there would be a tendency toward the establishment of equilibrium between the solution between the cells and the cell sap. Again, the growth of the organism between the cells with the consequent passage

of nutritive substances through the cell walls must exert an influence on the concentration of the cell sap. Then, too, the gelatinous material making up the bacterial cell walls certainly possesses considerable power of imbibition.

Further it has previously been pointed out that *Pseudomonas citri* exerts a strong diastatic activity when grown on potato cylinders. The production of this enzym has also been demonstrated by growth on starch agar prepared according to the method described by Crabill and Reed (4). Within a week a clear halo around the edge of the bacterial colony is formed on this substratum, thus making a striking ocular demonstration of dissolution of starch by the canker organism. If diastase, secreted by this organism, is readily diffusible through the cell walls, and it is reasonable to suppose that it is, it can convert the relatively insoluble starch into more soluble carbohydrates and thus increase the osmotic pressure of the cell sap.

It is not impossible that these several causes of increased osmotic pressure operating conjointly or separately may so profoundly modify the imbibitory properties of certain colloidal substances within the cells that their affinity for water is in consequence greatly increased.

No attempt has been made to determine the isotonic coefficient of the cell contents of the enlarged cells, but for the reasons just mentioned it is believed to be greater than that of normal cells.

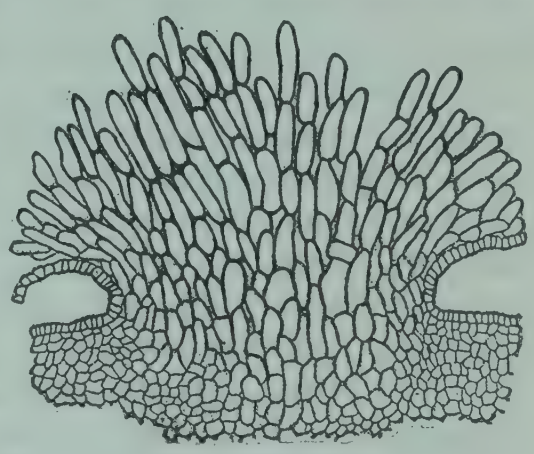

FIG. 7.—Cross section in outline of a spongy canker on the rind of a fruit of *Citrus decumana*, showing ruptured epidermis and hypertrophy of the rind tissues, the cells of which are loosely attached.

### DISINTEGRATION OF THE TISSUES

An attempt has been made to gain certain information relative to the organisms involved in the disintegration of cankerous tissues, together with the nature of their activity on this tissue. It was previously pointed out that a species of Phoma is commonly associated with Citrus canker. Two other species of fungi belonging to the genera Gloeosporium and Fusarium are also sometimes present. Since certain bacteria and fungi are known to possess the power of hydrolyzing cellulose (13, 15), of which complex substance cell walls are largely constituted, an effort has been made to study the action of the organisms associated with canker upon pure cellulose. For this purpose cellulose agar was prepared according to the following method. Schweitzer's reagent was first made by adding ammonium chlorid and then an excess of sodium hydrate to a solution of copper sulphate. The blue precipitate thus formed was washed, pressed on a cloth filter, and dissolved in ammonium hydrate (sp. gr. 0.92). In this solvent 15 gm.

of sheet filter paper were dissolved, the solution was diluted about 10 times with water, and the cellulose was precipitated with a 15 per cent solution of hydrochloric acid. After considerable dilution the mixture was filtered, and the residue was washed repeatedly with water to remove all copper and chlorin. This residue was added to an agar medium consisting of agar, 10 gm.; monopotassium phosphate, 1 gm.; magnesium sulphate, 1 gm.; sodium chlorid, 1 gm.; ammonium sulphate, 1 gm.; calcium nitrate, 0.5 gm.; and the whole was made up to 1,000 c. c.

Poured plates of cellulose agar were made during May, inoculated with *Pseudomonas citri*, *Phoma* sp., *Gloeosporium* sp., and *Fusarium* sp., and incubated at room temperature. All grew poorly and none of the fungi fruited on this medium. There was no evidence of the production of cellulase except by *Phoma* sp. Within two weeks this organism had formed clear translucent halos as shown in Plate IX, figure 5, indicating that the cellulose had been hydrolyzed. Even though *Phoma* spp. strongly dissolve paper cellulose, they may not behave in this manner toward cell walls of *Citrus* spp., since other carbohydrates present would be more readily available than cellulose.

A further effort has been made to determine what other enzyms are secreted by these organisms and what part they might consequently play in the destruction of the tissues. Accordingly, Knop's mineral nutrient solution was prepared for use as a stock solution. This stock solution was then tubed and sterilized. To one set of these tubes of Knop's solution starch was added, to another saccharose, and to another maltose. They were then set aside and tested to determine whether they were sterile. It had previously been determined that sterilization subsequent to adding the carbohydrates resulted in a certain amount of conversion of these carbohydrates. When it was determined that they were sterile, four sets of four tubes each were taken of each of the nutrient solutions. Three tubes in each set of four were inoculated with pure cultures of one of the four organisms mentioned above and one tube in each set was left as a check. After 10 days the solutions were tested, with the following results: Fehling's solution showed a strong reduction in the starch solutions in which *Pseudomonas citri* and *Phoma* sp. had been grown, showing the production of diastase. There was no change in the checks nor in the solutions in which the other organisms were grown.

Inversion of saccharose, as evidenced on the reduction of Fehling's solution, had been accomplished in the solutions in which *Phoma* sp. and *Fusarium* sp. had been grown, indicating the presence of invertase. Positive tests for dextrose or glucose were secured with Barfoed's reagent and with Nylander's reagent in these inverted saccharose solutions. Negative results were secured with the other organisms and with the checks.

*Phoma* sp. alone seemed to have any action on maltose. Inversion into dextrose was shown by positive tests with Barfoed's reagent.

Negative tests for lipase production were secured in the case of each of the four organisms.

From the foregoing tests it is seen that *Phoma* sp. secretes cellulase, diastase, invertase, and maltase, and must therefore be regarded as very destructive to the carbohydrate material of diseased tissues. Cellulase very probably aids in the destruction of the cell walls; diastase converts the starch into maltose and dextrin and then further acts on the dextrin. When a few drops of iodin were added to a starch solution in which *Phoma* sp. had grown, blue and red colors developed, indicating amylo- and erythro-dextrin. Maltase probably further reduces the maltose to dextrose.

It has also been found that *Phoma* sp. affects the acidity of the medium upon which it is grown. This was determined by growth in pure culture of the fungus on leaves and fruits of *Citrus trifoliata*. This material was first macerated by passing it through a meat chopper. Thirty-gm. samples of ground leaves and of fruits were then placed in 250 c. c. Erlenmeyer flasks and were sterilized in an autoclave. After sterilization some were inoculated with *Phoma* sp. from pure cultures and others left as checks. A copious white growth occurred on those which had been inoculated. After a month 150 c. c. of distilled water were added to each of the flask cultures and to the checks. The flasks were then heated on a water bath for 30 minutes, the liquid filtered through asbestos, and 25 c. c. of the filtrate taken for titration, using *N/20* sodium hydroxid, with litmus paper as an indicator. The following is representative of the results obtained: 7.1 c. c. of *N/20* sodium hydroxid neutralized 25 c. c. of the filtrate from the leaves in the check flask and 9.8 c. c. that from the fruits in the check flask. The filtrate from the leaves upon which *Phoma* sp. had been growing was neutral to litmus, and that from the fruits required 1.3 c. c. of *N/20* sodium hydroxid to neutralize it. From this it is concluded that *Phoma* sp. is able to utilize the organic acids as a source of food, a condition contrary to that which Hawkins (7) found in a study of the chemical changes produced by the brown-rot fungus on peaches.

### TAXONOMY OF THE FUNGUS

An effort has been made definitely to assign this species of Phoma to one of the numerous species of the form genera Phoma and Phyllosticta, which have previously been described as occurring on parts of *Citrus* spp. The pycnidia of the species under consideration are globose, ostiolate, 100 to 150$\mu$ in diameter (Pl. XI, fig. 5, 6) and wholly or partially embedded within the cankerous tissue. The pycnidial walls are thin, being thickest around the ostiolum, and are very similar in color to the corky brown host cells. The conidia are elliptical or oblong in outline,

hyalin, and 9 to 12 by 3 to 4μ. They germinate within 24 hours in water or in a variety of culture media. A white mycelial growth is produced on bean agar. Pycnidia are readily formed on agar (Pl. XI, fig. 3) modified by the addition of a water extract from corn meal, rice, cowpeas, orange stems, etc. (fig. 8).

The fungus, furthermore, was very probably introduced into the United States similtaneously with *Pseudomonas citri*. It is impossible to determine the position of this organism among previously described species, since it has been found to be morphologically not unlike several of them. Its relation to the production of Citrus canker is definitely established as a result of this study. Then, too, no particular difficulty would be experienced by other investigators in identifying it because of its association with Citrus canker. In view of these facts it seems well to describe it as a new species with the following brief technical diagnosis:[1]

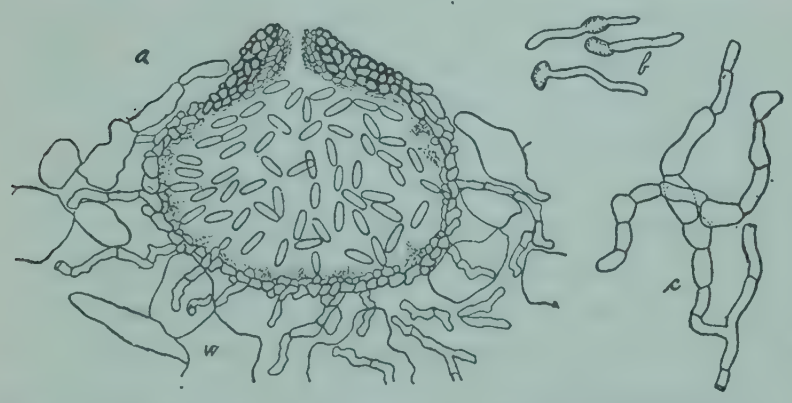


FIG. 8.—*a*, Cross section of a pycnidium of *Phoma socia* from a grapefruit leaf. This material was fixed in chromo-acetic acid, embedded in paraffin, sectioned, and stained in saffranin and gentian violet. Drawing outlined with the aid of a camera lucida. *b*, Germination of conidia of *Phoma socia* after 24 hours in water. *c*, Mycelium of this fungus in old cultures.

**Phoma socia**, n. sp.

Pycnidia irregularly distributed, globose, wholly or partially embedded, 100 to 150μ in diameter; walls thin, corky brown in color, thickened only around the ostiolum, which opens centrally; conidia continuous, elliptical or oblong, hyalin, 9 to 12 by 3 to 4μ.

Occurs in the cankers produced by *Pseudomonas citri* on living leaves and branches of *Citrus trifoliata*, *C. nobilis*, and *Fortunella* sp. and on living leaves, branches, and fruits of *C. decumana* and *C. aurantium*.

## ACIDITY AND RESISTANCE TO CANKER

It is generally conceded by both nurserymen and growers and has been substantiated by the field observations of the writer that Satsuma oranges are not as susceptible to Citrus canker as grapefruit. This difference may be noted when both species are grown in locations where they are equally exposed to infection. The tolerance of bacteria to acidity has been found to be relatively low. Resistance to certain fungus diseases, as, for example, the resistance of hard wheat to rust, has been found (3)

[1] **Phoma socia**, sp. nov.

Perithecis irregulariter distributis, globosis plus minusve immersis, 100 to 150μ diam.; contextu membranaceo, corticale-brunneo, cum cellulis circa ostiolum pseudoparenchymaticis, centro perforatis; sporulis continuis, ellipticis v. oblongis, hyalinis 9–12 × 3–4μ. Hab. in foliis ramisque, vivis *Citri trifoliatae*, *C. nobilis* et *Fortunellae* sp. et quoque in foliis, ramis fructibusque *C. decumanae* et *C. aurantii*. Socia adest *Pseudomonas citri* Hasse.

to be correlated with the acidity of the cell sap. Because of these several facts, an effort has been made to determine whether the difference in susceptibility between Satsuma oranges and grapefruit can be accounted for on the basis of difference in acidity. Leaves collected from plants growing in the greenhouse were used in these tests. The leaves were finely macerated by trituration; distilled water was then added to make a volume equaling 200 times the weight of the finely ground leaves; phenolphthalein was added as an indicator; and the acids present in the sample were titrated with *N/10* sodium hydroxid. This method is open to criticism where absolutely accurate determinations are sought, but is regarded as satisfactory in indicating relative differences. Considerable variations in acidity of the same species were noted, dependent largely upon the cessation of photosynthetic activity at night. Greater acidity, as would be expected, occurred in samples collected early in the morning. Representative results of these tests, however, are shown in Table I.

TABLE I.—*Acidity of oranges and grapefruit*

| Variety. | Wet weight of tissue. | Quantity of *N/10* sodium hydroxid to neutralize 1 gm. wet weight of tissue. | | Percentage of moisture in sample. | Percentage of total acidity based upon average water content. |
|---|---|---|---|---|---|
| | | Actual. | Average. | | |
| | *Gm.* | *C. c.* | *C. c.* | | |
| Satsuma (old) | 3.62 | 1.0359 | 1.0184 | 60.2 | 1.691 |
| | 3.06 | .9967 | | | |
| | 4.14 | 1.0406 | | | |
| | 4.17 | 1.0431 | | | |
| | 2.92 | .9760 | | | |
| Satsuma (young) | 2.60 | .9423 | .9735 | 67.8 | 1.465 |
| | 2.90 | 1.0172 | | | |
| | 2.55 | .9609 | | | |
| Grapefruit (old) | 2.71 | .8672 | .8787 | 59.0 | 1.490 |
| | 2.53 | .8695 | | | |
| | 1.66 | .8434 | | | |
| | 2.11 | .9005 | | | |
| | 1.95 | .9128 | | | |
| Grapefruit (young) | 1.97 | .8629 | .8634 | 79.9 | 1.080 |
| | 2.03 | .8620 | | | |
| | 1.56 | .8653 | | | |

The leaves of Satsuma oranges are consistently higher in acid content than those of grapefruit, since the former require 1.0184 c. c. of *N/10* sodium hydroxid to neutralize 1 gm. of wet weight of leaf tissue, young Satsuma leaves, 0.9735 c. c., old grapefruit leaves, 0.8787 c. c., and young grapefruit leaves, 0.8634 c. c. When the acidity of the cell sap is computed on the basis of the total moisture content of the leaves, it is found to be 1.691 per cent for old Satsuma leaves, 1.465 per cent in those of young Satsumas, 1.490 per cent in those of old grapefruit, and 1.080

per cent in those of young grapefruit. It will be recalled that bacterial growth occurs on artificial media rendered acid by hydrochloric or citric acid when a sufficient amount of acid has been used to make the acidity of the media 2 per cent. The acidity of the leaf tissue is therefore not sufficient to inhibit the growth of the canker organism and is not regarded as sufficient to account for the difference in susceptibility. No determinations have been made of the kinds and relative amounts of the several organic acids in the tissues of the two species. Until this is known there still remains the possibility of a correlation between susceptibility to canker and acidity.

## CHEMICAL CHANGES IN CITRUS LEAVES BROUGHT ABOUT BY CITRUS CANKER

Little attention has been given by the biochemist to the chemical transformations occurring in diseased plant tissues. Such studies would no doubt throw a flood of light upon the intimate relationship of parasite and host and would materially contribute to our knowledge of the nature of parasitism. The literature dealing with the chemical changes induced by plant pathogens is more or less fragmentary, mainly because of the inexact state of our knowledge regarding the separation and quantitative estimation of the various compounds occurring in plant tissues. An historical résumé of this literature has therefore been purposely omitted. However, among the recent excellent papers along this line may be mentioned the work of Hawkins (7) upon the changes in peaches induced by the brown-rot organism, *Sclerotinia cinerea*. He found in brown-rotted tissues an increase in acid content, a decrease in certain alcohol-soluble substances, a decrease in the total sugar content, and practically a disappearance of the cane sugar. It was with the view of determining something of the changes produced by Citrus canker that this portion of the investigation was undertaken.

Diseased and healthy leaves were taken from grapefruit trees affected with Citrus canker. Circles of diseased tissue and tissue from healthy leaves were excised with a cork borer. These leaf circles were then triturated in a mortar until the material was finely divided, their wet weight determined, 27.25 gm. in each case, and preserved in such volume of 95 per cent alcohol that the alcohol concentration of the mixture was 85 per cent. This concentration could not be accurately made until it had been determined that the moisture content of normal leaves was 61.69 per cent and that of diseased leaves 61.57 per cent. The material was then set aside for two weeks and was shaken occasionally to permit the gradual extraction of the cold alcohol-soluble portions. The method followed subsequently was based upon those devised by Koch (9, 10, 11, 12) for use in the quantitative chemical analysis of animal tissues.

This method consists essentially in the separation of the material into three fractions. Fractions 1 and 2 consist of the soluble portion extracted by the action of alcohol, ether, and water, and fraction 3 consists of the insoluble residue. Fractions 1 and 2 are separated by lipoid precipitation. The former fraction contains precipitated lipoids, while the latter contains all nonlipoid materials, soluble in alcohol, ether, and water. Instead of the modified Wiley extraction apparatus employed by Koch and his pupils, a rubber analysis extraction apparatus (8) has been employed. Extractions in this apparatus, like those with the modified Wiley apparatus, are carried out at the boiling point of the solvent.

In making the first alcohol extraction the preserved material was transferred to Schleicher and Schüll extraction thimbles, previously fitted into the siphon cups of the extraction apparatus. The preserving liquid was then filtered through these thimbles. Perforated porcelain plates or filter paper cut to fit were then used as covers over the material in the thimbles.

Extraction for 12 hours with redistilled 95 per cent alcohol followed. The alcohol was changed two or three times during this extraction in order to prevent possible decomposition of extracted materials in the boiling alcohol. The tissue was pressed to remove the excess alcohol, and an ether extraction was made. This extraction was continued for 12 hours for the purpose of facilitating the subsequent powdering of the tissues. The material was then removed from the extraction thimbles to a mortar and was ground to a powder. This powder was placed in a stoppered flask with a volume of distilled water equaling twice the fresh weight of the material and was boiled on a steam bath for two hours. Warm absolute alcohol was added in a sufficient quantity to bring the alcohol content of the whole up to 90 per cent. The mixture was warmed on the bath, with repeated shaking, and set aside until the following day. It was then filtered through the original extraction thimbles and extracted for 12 hours with 95 per cent alcohol. At the close of this extraction the residue in the cups (fraction 3) was transferred to previously weighed porcelain crucibles and dried to constant weight in an oven at 100° C. By this procedure the alcohol, ether, and water-soluble portions (fractions 1 and 2) were separated from the insoluble portion (fraction 3).

In further preparing the soluble portions of the material for analyses they were combined only after the ether-soluble portion had been heated on a water bath until the odor of ether could no longer be detected. A little alcohol was added from time to time to take up the materials left behind by the loss of ether by evaporation. In pouring the solutions together a precipitate appeared which was rendered soluble by the addition of sufficient hot water to bring the alcohol concentration down to 70 per cent. The solution was then made up to 2,000 c. c., 200 c. c. of which were taken for the estimation of solids. The remainder was

evaporated at 75° C. to a sirupy consistency or until all the alcohol had evaporated and the sirupy mass was emulsified with warm water. This emulsion was placed in a stoppered volumetric flask, shaken with 20 c. c. of chloroform, 10 c. c. of hydrochloric acid were slowly added, and then it was made up to a given volume by the addition of water. The flask was then placed for 48 hours in running water under the hydrant to facilitate the precipitation of the lipoids in the chloroform. Filtration followed, the filtrate constituting fraction 2, and the lipoid precipitate on the filter paper fraction 1. The precipitate was then taken up with a large volume of hot, 95 per cent alcohol, and kept on a water bath at 75 C., until all of the chloroform was driven off. The volume was then increased to a convenient amount, and aliquot parts taken for analyses.

The analysis of fraction 3 included (*a*) total phosphorus, (*b*) total nitrogen, (*c*) cellulose, (*d*) carbohydrate after hydrolysis, (*e*) ash, (*f*) total solids; the analysis of fraction 2 included (*a*) dry weight and ash made upon an aliquot part, (*b*) total sugars before and after hydrolysis, (*c*) total nitrogen, (*d*) phosphorus, (*e*) solids; while the analysis of fraction 1 included only (*a*) total solids, (*b*) phosphorus, (*c*) nitrogen, since the total weight of the lipoidal material from the two samples differed by 1 mgm. only and since the amounts were too small to admit of accurate separation.

The determinations of phosphorus were made upon aliquot parts by the Pemberton-Neuman method described by Mathews (14, p. 893–895).

The total nitrogen was determined upon all fractions by the employment of the Gunning-Arnold modification of the Kjeldahl method. No determinations were made of fatty acids.

In fractions 2 and 3 the carbohydrate determination included reducing sugars, total sugars, and cellulose. Prior to the determination of reducing sugars, the solution was freed from organic acids, tannins, and other substances capable of affecting reduction by Fehling's solution. This was accomplished by treatment with lead subacetate in excess, after which the solution was diluted, filtered, and saturated sodium sulphate was added to precipitate the excess of lead. The clear filtrate was then diluted, and an aliquot part taken for the determination of reducing sugar by the Bertrand volumetric method. The reducing sugar was calculated as dextrose by the Munson and Walker tables.[1] Another aliquot part of the solution, a part of which had been used for the determination of reducing sugars, was used upon which to determine the total sugars. This was hydrolyzed by the addition of concentrated hydrochloric acid, following which the solution was kept on a water bath at 69° to 70° C. for 10 minutes. It was then cooled, neutralized with 40 per cent sodium hydroxid, and the sugar determined as invert sugar by the volumetric permanganate method.

[1] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 241–251. 1908.

Cellulose determinations in fraction 3 were made in duplicate with accordant results by employing Schweitzer's reagent in one case and a solution of zinc chlorid in hydrochloric acid in the other.

Polysaccharids in fraction 3 were estimated as dextrose after 2.5 hours' hydrolysis in a reflux condenser using 2.5 per cent hydrochloric acid.

In Table II are given the fresh weights of normal and cankerous Citrus tissue, moisture content, dry weight, and alcohol-ether soluble and insoluble portions.

TABLE II.—*Analysis of normal and cankerous tissue of grapefruit leaves*

| Item. | Normal tissue. | Cankerous tissue. |
|---|---|---|
| | *Gm.* | *Gm.* |
| Fresh weight | 27. 250 | 27. 250 |
| Moisture | 16. 799 | 16. 796 |
| Dry weight | 10. 451 | 10. 454 |
| Total alcohol-ether: | | |
| Soluble | 4. 270 | 4. 300 |
| Insoluble | 6. 181 | 6. 154 |

In this composite table it is strikingly significant that only slight differences are apparent. The moisture content of normal tissue is slightly greater than that of cankerous tissue, and there is, of course, a corresponding decrease in dry weight. The greater amount of alcohol-ether soluble material occurs in cankerous tissues with a lesser amount of alcohol-ether insoluble substance. The differences represented herein would have little or no value in themselves if it were not that they were obtained by the use of a refined method of analysis primarily intended to permit the discovery of changes not indicated by ordinary methods. Studies of the intricate relation of parasite and host have proceeded far enough to indicate that large changes in composition of the host are not to be expected, but rather that transformations have been produced which, though minute in amount, profoundly affect the metabolism of both parasite and host. Table III gives in detail the results of the several steps in this analytical procedure.

TABLE III.—*Analyses (in grams) of normal and cankerous grapefruit leaves*

| Item. | Fraction 1. | | Fraction 2. | | Fraction 3. | | Totals. | |
|---|---|---|---|---|---|---|---|---|
| | Normal. | Diseased. | Normal. | Diseased. | Normal. | Diseased. | Normal. | Diseased. |
| Dry weight | 0. 781 | 0. 780 | 3. 479 | 3. 520 | 6. 181 | 6. 154 | 10. 441 | 10. 454 |
| Nitrogen | . 0196 | . 686 | . 1190 | . 1120 | . 105 | . 0818 | . 2436 | . 2624 |
| Phosphorus | . 0123 | . 0114 | . 021 | . 0225 | . 0101 | . 0131 | . 0434 | . 0470 |
| Reducing sugars | ..... | ..... | 2. 008 | . 806 | ..... | ..... | 2. 008 | . 806 |
| Sugar after acid hydrolysis | ..... | ..... | . 387 | . 284 | . 573 | . 370 | . 960 | . 654 |
| Polysaccharids (soluble) | ..... | ..... | ..... | ..... | . 2087 | . 1273 | . 2087 | . 1273 |
| Cellulose | ..... | ..... | ..... | ..... | . 859 | . 843 | . 859 | . 843 |
| Ash | ..... | ..... | ..... | ..... | . 041 | . 041 | . 041 | . 041 |

It should be stated with reference to the data presented in Table III that the figures given are the weights in grams of the several constituents as determined by employing 27.25 gm., fresh weight, of healthy and of cankerous tissue. Since the two samples differed by only 3 mgm. in dry weight and since the figures, to be directly comparable, should be based on dry weights in each case, a correction of 0.24 per cent should be applied to the analyses of diseased tissue. As this is insignificant, the data are regarded as referable, and the corrections have not been applied.

Because of the presence of certain enzyms, of which mention has been made earlier in this paper, it is to be expected that the changes of greatest magnitude would occur in the carbohydrates. That such is the case is obvious when one notes in the totals given in Table III a reduction of all classes of carbohydrate in cankerous tissue. Thus, in equal quantities of fresh material the amounts of reducing sugar are found to be as 5 to 2, the total sugars as 3 to 2, and the polysaccharids as 5 to 3 when normal and diseased tissues are compared. Because of the ease with which they are available to the invading organisms, the reducing sugars are probably the most strongly attacked. After acid hydrolysis the normal tissue shows more reducing sugar than the diseased, both in the alcohol-ether soluble and alcohol-ether insoluble fractions. This means that there is also a less amount of the higher soluble carbohydrates, disaccharids, in diseased tissues and that they too are more easily available than the polysaccharids. The ratio of disaccharids in normal and cankerous tissue in the alcohol-ether soluble and alcohol-ether insoluble portions is as 3 to 2 and 5 to 3, respectively.

There is also a slight but significant decrease in the amount of cellulose found in diseased tissues. Although the difference in total cellulose in the normal and diseased tissues is slight, the results given are representative of a considerable number of determinations in which two standard methods were employed and in which the lesser amount of cellulose was invariably found in the diseased tissue. Experimental error has thus been eliminated and the results indicate a slight but unmistakable destruction of cellulose by the invading organisms.

The polysaccharids were determined in fraction 3 after 2.5 hours acid hydrolysis. They were found to be present in normal tissues and diseased tissues in the same proportion, 5 to 3, as were the disaccharids. There has therefore been a corresponding reduction and utilization of both di- and poly-saccharids by the invading organisms.

In the alcohol-ether insoluble fraction the amounts of nitrogen found for normal and diseased tissue were 0.105 and 0.0818 gm., respectively. If the conventional factor for these figures, 6.25, is employed, 0.654 and 0.511 gm. are obtained as the protein content of normal and diseased tissues, respectively. The protein content of diseased tissue has therefore been reduced 78.16 per cent. One should therefore expect to find a

very material increase in the nitrogen of the alcohol-ether soluble portion of the diseased tissue. This expectation is realized, since the nitrogen figures for the soluble portions are 0.1386 gm. for normal and 0.7806 gm. for diseased tissue. This represents an increase in the diseased tissue of 37.52 per cent over the healthy tissue. This increase in the soluble portion indicates a decomposition of the complex nitrogenous compounds resulting in the formation of peptones and amino acids soluble in alcohol and ether. This difference in nitrogen content of the alcohol-ether soluble portion takes an added significance when the nitrogen content of fraction 1 and that of fraction 2 are examined separately. It will be recalled that the nitrogen of fraction 2 represents those portions of the nitrogenous constituents extracted by alcohol and ether which are readily soluble in water after the combined extract has been evaporated to a paste. They are, therefore, amino acids and polypeptids. It will further be recalled that fraction 1 is obtained from the watery solution of the alcohol-ether soluble extract by chloroform precipitation and is therefore lipoid nitrogen. The slight decrease in nitrogen in fraction 2, when normal and diseased tissue are compared, is accompanied by an enormous increase, amounting to 250 per cent in the lipoid nitrogen of fraction 1.

These differences in nitrogen content of the several fractions lend themselves to two possible explanations. The first and most obvious interpretation of the results is that the changes produced by the invading organisms in the proteins of the host result in the formation not of amino acids and other end products of protein decomposition but in the production of complex intermediate substances. The other explanation is based upon the fact that the bacteria themselves derive the nitrogen necessary for the building of their own proteins as well as for the formation of their cell walls from the proteins of the host. Concurrently with the reduction of the protein of the host to simpler forms a series of metabolic processes is occurring within the invading organism which involves the synthesis of these simple nitrogenous compounds to more complex ones. The changes in nitrogen content of the several fractions of the diseased tissue are therefore the result of both analytic and synthetic processes. At present it is impossible to employ any methods, as none have been devised, which will indicate what the end products of decomposition of the host proteins by the invading organism are, since the formation of these products is accompanied by their concomitant utilization in the manufacture of new compounds peculiar to the body of the parasite.

The total phosphorus in the diseased tissues is greater in amount in fractions 2 and 3 than in the normal tissues. Were the changes in the diseased tissue purely katabolic, it would be expected that there would be a material increase in water-soluble phosphorus derived from the

decomposition of neucleoproteins. On the contrary, the phosphorus of fraction 3 shows an increase of 30 per cent, that of fraction 2 an increase of 20 per cent, and that of fraction 1 a decrease of about 7 per cent. The increase in water-soluble phosphorus in fraction 2 indicates that decomposition processes are taking place, but the concomitant increase in phosphorus content in fraction 3 shows that such decomposition is accompanied by actual synthetic processes involving the use of phosphorus.

No difference appears between the two tissues in amounts of ash as shown in fraction 3. The ashing of fractions 1 and 2 gave unsatisfactory results and for this reason the figures are withheld.

It is evident from the foregoing statement of results that the significant changes brought about in diseased tissues concern carbohydrate and nitrogenous constituents. The concurrent disappearance of mono-, di-, and poly-saccharids from diseased tissues indicates that all the sucroclastic enzyms previously shown to be formed by the organisms in pure cultures are active in the host tissues and that the reducing sugars formed are utilized by the organisms as sources of energy. The results with nitrogen indicate that there is not an accumulation of the products of protein decomposition but that the destructive transformation of protein is accompanied *pari passu* by a utilization of the decomposition products in the anabolic processes of the organisms.

## AGENCIES CONCERNED IN DISSEMINATION OF CITRUS CANKER

Definite experimental data are wanting on the agencies by which Citrus canker is spread. If we judge, however, from field observations and from a knowledge of other bacterial plant diseases, it is evident that rain and dew are important factors in carrying the disease to unaffected leaves, twigs, and fruits of trees in which the diesase is already present. Man himself is a very important agent in effecting the distribution of canker from diseased trees to healthy trees near by. When in the cultural operations of budding, cultivation, picking, etc., he comes in contact with diseased trees and soon afterwards touches healthy ones, infection may result. The chances of infection are greatly increased if he comes in contact with newly formed cankers on the diseased trees, and if a film of moisture is present on the adjacent healthy trees which he may touch. The most plausible explanation of the introduction of Citrus canker into two groves which have come under the writer's observation is through the agency of man. The owners had visited groves in which canker occurred in order to acquaint themselves with the appearance of the disease. On returning home they examined certain of the trees in their own groves and these trees soon afterward developed canker lesions. Stirling (2) reports the transmission of the disease through handling diseased leaves prior to touching healthy ones. It is highly probable that

certain birds and insects also effect this contact of diseased with healthy parts and are therefore to be regarded as agents in dissemination of Citrus canker.

## CONTROL OF THE DISEASE

During the summer of 1914 those who had been attempting to solve the problem of controlling Citrus canker realized that it was an exceedingly difficult undertaking. Efforts were directed along three lines: Exclusion, protection, and eradication.

EXCLUSION.—Those interested in the welfare of the Citrus industry in Florida were the first to realize the serious nature of Citrus canker and that it had been introduced into the State from other States and from foreign countries. For these reasons a quarantine was imposed during the spring of 1914 to prevent the further introduction into Florida of Citrus trees and buds and thus of Citrus canker. Other of the Gulf States later in the season realized the jeopardy in which their Citrus growers' interests were placed and issued similar regulatory measures on the importation of shipments of Citrus stock. On January 1, 1915, a Federal quarantine was imposed to exclude the further importation of this disease into the United States. The agitation throughout the entire Citrus growing section of the Gulf coast attendant on the adoption of these regulations looking toward control by exclusion have so familiarized the growers with Citrus canker that it is unnecessary to advise the exercise of care in ordering trees to be used in setting out a Citrus grove. It is realized that in no case is it safe to purchase trees from nurseries in which this disease occurs.

PROTECTION.—Since certain fungicides have been successfully used in the control of various Citrus diseases a number of experiments were undertaken during the spring of 1914 to determine the effectiveness of these mixtures in the control of Citrus canker. A grove of badly diseased grapefruit was used upon which to make applications of Bordeaux mixture, ammoniacal copper carbonate, and soluble sulphur. Details of these experiments are withheld, since it was realized early in the summer that the application of these fungicides was without appreciable effect in the control of canker.

Again in the spring of 1915 another grove of grapefruit was selected in which to test the effectiveness of several fungicides in protecting the trees from infection by the canker organism. All visible signs of canker were carefully removed from the trees prior to the application of the mixtures. Bordeaux mixture, Bordeaux mixture and bichlorid of mercury (12 tablets in 3 gallons), Bordeaux mixture and formaldehyde (1 : 100), and a Bordeaux and lead arsenate mixture were employed. Applications were made on March 26, April 29, and May 14, and no new infections had developed on any of the sprayed or unsprayed trees by the last-named date. On May 27, however, new infections were apparent

and were equally numerous on sprayed and check trees. A number of growers have used various germicidal mixtures in attempts to find a preparation which could be successfully employed against Citrus canker. In no case have these efforts met with a sufficient degree of success so that their use in canker control can be recommended. When formaldehyde is used in sufficient strength to cause the death of the leaf tissues in a considerable area surrounding the cankers no viable organisms can be found in the cankerous tissues in many cases. They are still viable, however, in others, and it has also been found to be impossible to cause formaldehyde to penetrate sufficiently deep into old suberized limb cankers to kill the canker organisms. In the light of these tests and in the light of the ineffectiveness of sprays in the control of other plant diseases of bacterial origin, it is believed that there is little to be hoped for in the use of germicides for protection against Citrus canker.

ERADICATION.—The history of the work of eradication of Citrus canker, little of which has been published outside of the daily press, would in itself be voluminous, and it is not the present purpose to include it in this account. The early efforts toward the eradication of Citrus canker were confined to the removal of diseased parts in case the trees were only slightly diseased. When the trees were seriously affected, however, they were severely pruned, even though this necessitated the removal of nearly all of the branches. Pruned trees were then thoroughly sprayed with Bordeaux mixture. It was recommended that all the diseased parts which had been removed should be burned.

After a few months' trial it was seen that by this procedure the treated trees were still diseased. Further than this, adjacent trees had become diseased, although they were apparently healthy at the time efforts had been made to remove cankered leaves and branches from the trees near by.

Even when the work of removal had been done by skilled hands and when the trees had received several applications of some fungicide to protect the new growth they were still found to become cankered.

As a result of this it was decided during the summer of 1914 that only the complete destruction of the diseased trees by burning would be effective. As a result of this decision the eradication campaign was organized and a concerted, heroic effort is being put forth to stamp out Citrus canker from the Gulf States. The intelligent observance of the strictest sanitary precautions with reference to trees adjacent to those which are destroyed is necessary.

## SUMMARY

A serious disease, commonly known as Citrus canker, which affects species of Citrus and Fortunella, has within the past few years been introduced into Alabama and other of the Gulf States. It attacks fruits, leaves, twigs, and larger branches, producing characteristic cankerous

lesions. The primary cause of the disease is *Pseudomonas citri*, first isolated by Hasse from grapefruit and found to be pathogenic on grapefruit seedlings. This has been confirmed, and in addition an organism presenting the same cultural and physiological characters has been isolated from trifoliate and Satsuma oranges and lemons. No difficulty has been experienced in cross-inoculating the organism on McCarty and seedling grapefruit, Pineapple oranges, Satsuma oranges, and seedling trifoliate oranges. It grows readily on a variety of artificial media, and according to the studies made its group number is 221.3332513.

Infection occurs through natural openings and through wounds. The rapid spread of the disease is favored by the simultaneous occurrence of newly exposed cankerous cells and the presence of a film of moisture, especially on young parts of the plant. The bacteria occur for the most part between the cells of the host and cause them to become considerably hypertrophied. Little, if any, hyperplasia is believed to occur. This enlargement of the cells is caused by the dissolution of the middle lamellæ through enzym activity and by a modification of the host protoplast so that its osmotic pressure is increased. This increased pressure results from the presence of the parasite between the cells and from the passage of materials through the walls of the host, occasioned by the growth of the organism.

Besides *Pseudomonas citri*, fungi belonging to the genera Phoma, Fusarium, and Gloeosporium have been isolated from Citrus cankers. Of the fungi *Phoma* sp. alone was found to be notably active in the disintegration of the tissues. It is able by virtue of the secretion of specific enzyms to utilize the carbohydrates, cellulose, starch, maltose, and saccharose and causes also a decrease in acidity of invaded tissues. It is regarded as heretofore undescribed and is herein given the name "*Phoma socia*, n. sp."

The difference in susceptibility to Citrus canker of Satsuma oranges and grapefruit can not be accounted for on the basis of differences in total organic acids in the two hosts.

Comparative analyses of grapefruit leaves affected with Citrus canker and of healthy leaves shows that there has been in diseased leaves a decrease in all of the soluble and insoluble carbohydrates due to their utilization by means of sucroclastic enzyms secreted by the canker organisms. Apparently a decomposition of the host proteins occurs concurrently with their synthesis in the metabolism of the parasite proteins, and there results a slight increase in total nitrogen in diseased tissues. The slight increase in phosphorus in diseased tissues is accounted for in the same manner as that in nitrogen, since they appear to be correlated. No differences in ash were found in fraction 3, and the dry weight of diseased tissues was slightly greater than that of normal.

Rain and dew are important agencies in the dissemination of Citrus canker. Any other agencies, of which man is probably the most im-

portant, which effect a contact of diseased parts with healthy parts, are to be recognized as carriers.

In efforts to control the disease quarantine measures have been passed, thus preventing its further introduction from foreign localities and from any one of the Gulf States to any other of them. The use of spray mixtures indicates that they are not to be regarded as remedial measures of appreciable value in canker control; nor will their use protect healthy trees adjacent to diseased ones from infection.

Successful eradication seems possible, but only when the work of destruction of diseased trees is thoroughly done, with the observance of proper sanitary precautions.

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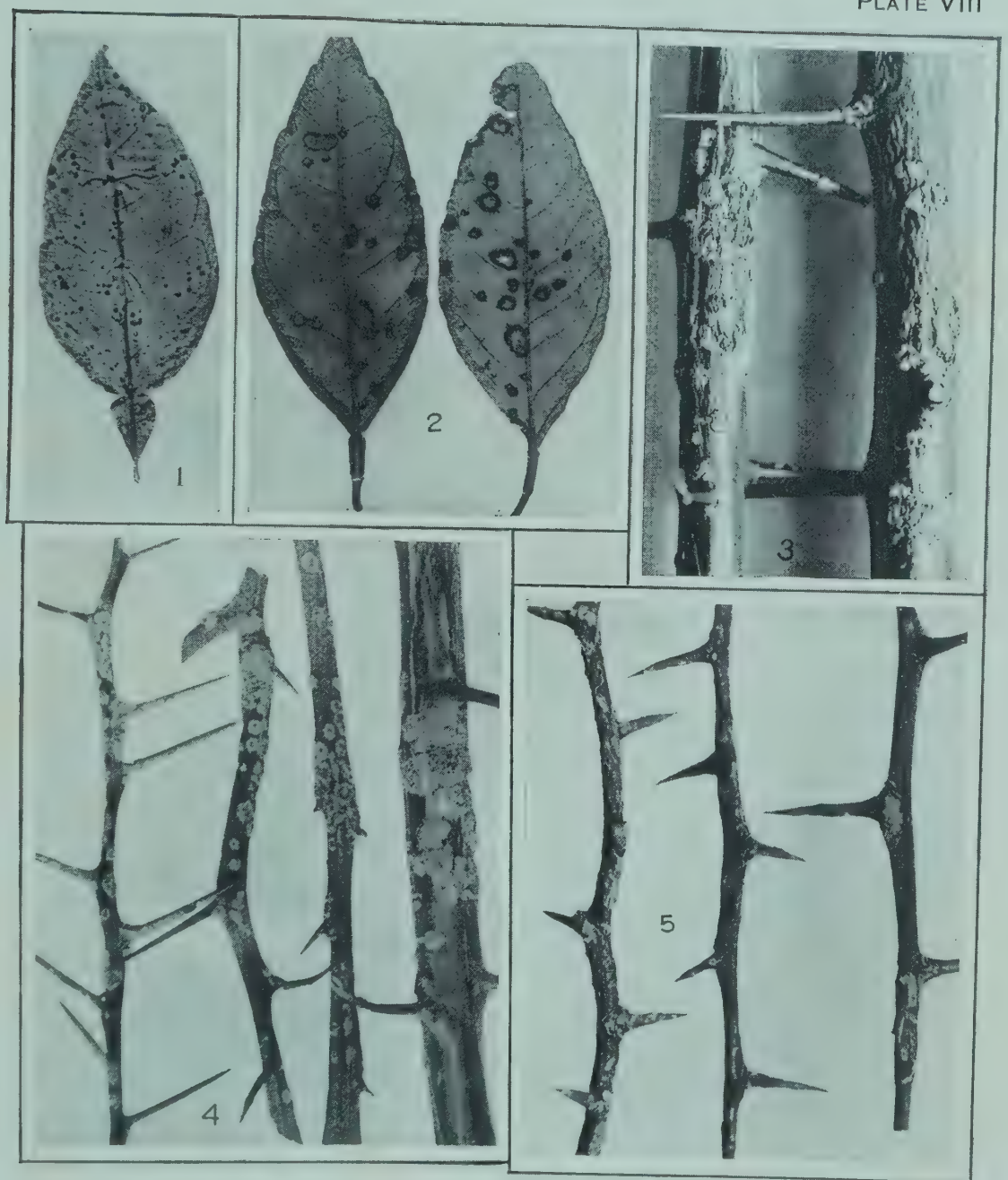
## PLATE VIII

Fig. 1.—Grapefruit leaf showing young Citrus cankers.
Fig. 2.—Old Citrus canker on Satsuma leaves.
Fig. 3, 4.—Seedling grapefruit branches affected with Citrus canker.
Fig. 5.—Severe canker infection of branches of *Citrus trifoliata*.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## PLATE IX

Fig. 1.—View of lower side of leaves of seedling grapefruit artificially inoculated with *Pseudomonas citri.*

Fig. 2.—Top view of plant shown in figure 1.

Fig. 3.—Spongy white cankers on leaf and twig of seedling grapefruit produced by artificial inoculation. The plants were continuously kept under a bell jar in a humid atmosphere.

Fig. 4.—Citrus canker on Satsuma leaves resulting from artificial inoculation with *Pseudomonas citri.*

Fig. 5.—Photomicrograph of section of young, open canker on grapefruit.

## PLATE X

Fig. 1.—Natural Citrus canker infection on leaves of *Citrus trifoliata.*

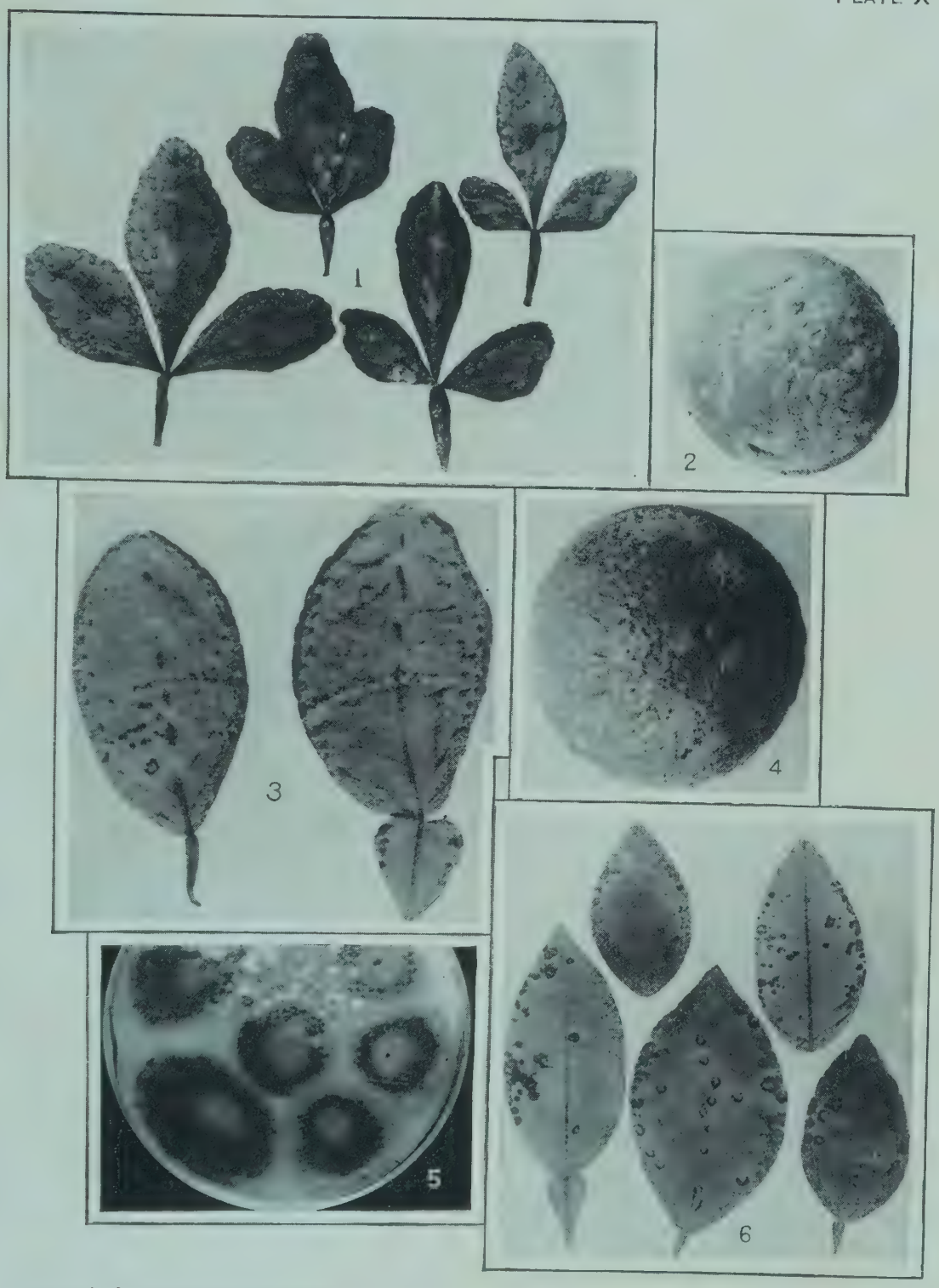
Fig. 2.—Mature cankers on fruit of *Citrus decumana* (courtesy of Dr. E. W. Berger).

Fig. 3.—Canker on seedling grapefruit leaves, entrance having been effected through abrasions made by thorns.

Fig. 4.—Young spongy cankers on fruit of *Citrus decumana.*

Fig. 5.—*Phoma socia* on cellulose agar showing dissolution of cellulose.

Fig. 6.—Mature cankerous areas on leaves of Duncan grapefruit.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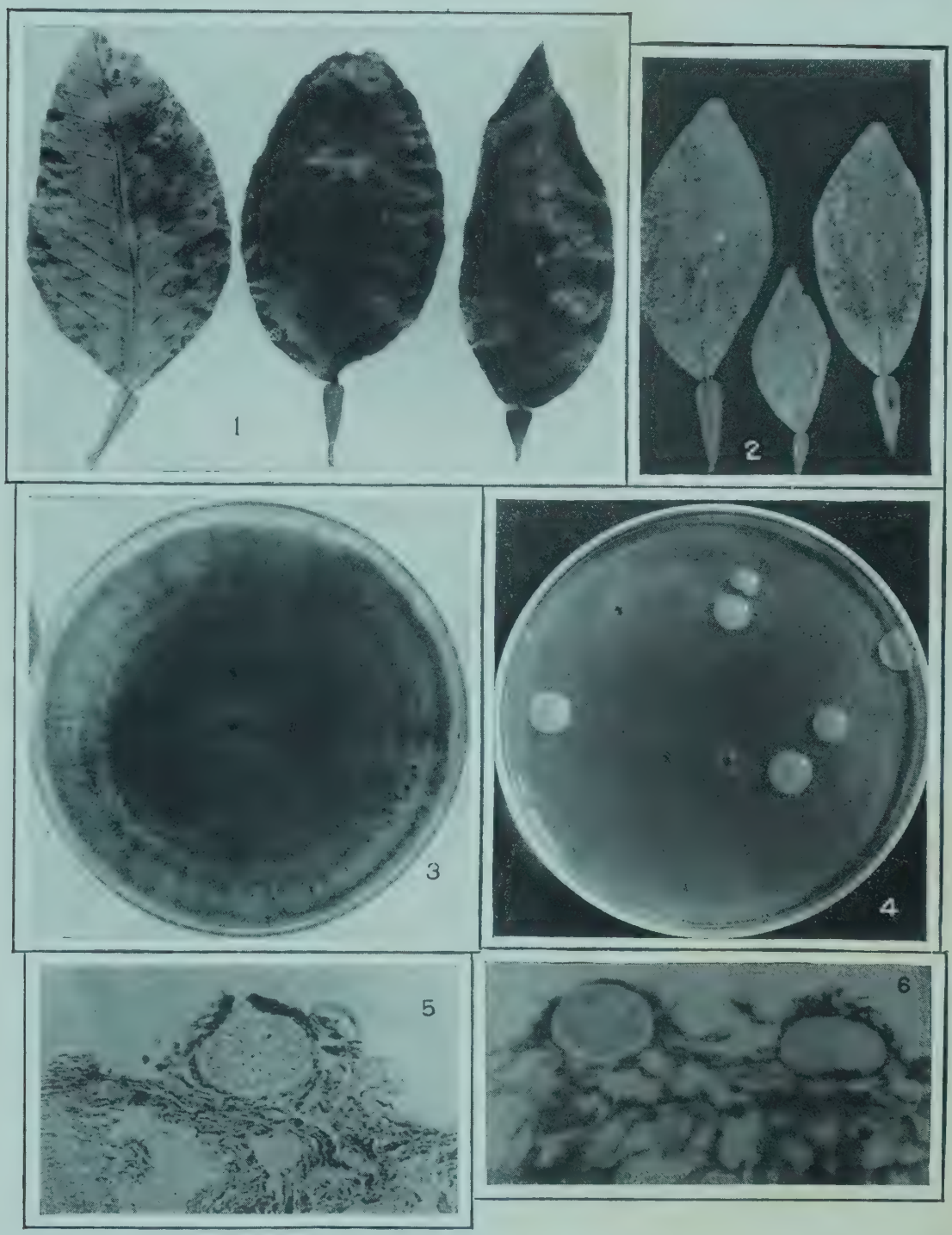
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## PLATE XI

Fig. 1.—Cankers on old grapefruit leaves which have enlarged during the second growing season.

Fig. 2.—Citrus canker resulting from immersion of leaves in a bacterial suspension. Lesions involving a large part of the lower leaf surface are thus formed.

Fig. 3.—Culture of *Phoma socia* showing pycnidial formation in concentric rings.

Fig. 4.—Dilution poured plate of *Pseudomonas citri* on green-bean agar. The spots on the colonies are the reflection of the windows of the room in which the exposure was made. Colonies 14 days old, the last 5 of which days the plates were kept in an ice chest at a temperature of about 55°.

Fig. 5.—Photomicrograph of pycnidium of *Phoma socia* taken in reflected sunlight.

Fig. 6.—Photomicrograph of pycnidia of *Phoma socia* taken in diffuse light.

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# DETERMINATION OF STEARIC ACID IN BUTTER FAT[1]

By E. B. HOLLAND, *Associate Chemist*, and J. C. REED and J. P. BUCKLEY, Jr., *Assistant Chemists, Massachusetts Agricultural Experiment Station*

## INTRODUCTION

Oils and fats are composed largely of neutral glyceryl esters together with small amounts of free fatty acids and unsaponifiable matter. Formerly the esters were considered simple glycerids, compounds of glycerol and three radicals of the same fatty acids. At present the opposite view seems to prevail and mixed glycerids are said to predominate in most products. The subject is controversial and difficult of solution. The constituents would be the same, however, in either case, whether combined as simple or complex molecules. The object of a technical examination of oils and fats is to isolate, identify, and determine the various fatty acids, glycerol, and unsaponifiable bodies, although, as Lewkowitsch asserts, this is not attainable in the present state of our knowledge. Certain progress has been made in determining different constituents of fats by indirect methods, such as iodin absorption, acetyl number, and molecular-weight calculations. Direct methods of fractional distillation, crystallization, and solubility of various salts have not, as a rule, proved sufficiently discriminative for quantitative use.

Fatty acids constitute about 95 per cent of most oils and fats and characterize the products to a large extent. The necessity of accurate methods for the quantitative determination of these acids has long been recognized not only from the standpoint of pure science but especially in physiological studies having as the object the measurement of the effect of different food groups on the production of body and milk fats. Many methods have been proposed since the publication of the work of Chevreul nearly 100 years ago, but few, if any, have met with general approval. After several years' investigations of the Partheil and Ferie method (7),[2] which proved unsatisfactory in the authors'[3] hands, a study of methods for determining stearic acid in butter fat was undertaken.

[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

[2] Reference is made by number to "Literature cited," p. 113.

[3] Mr. Reed was associated with the senior author in the earlier stages of the work and Mr. Buckley in the later.

## EARLIER INVESTIGATIONS

For the separation of stearic from other fatty acids, David (1) recommended a special alcohol and dilute acetic-acid solution saturated with stearic acid at 15° C., in which solution oleic acid was shown to be soluble.

The Hehner and Mitchell (3) method for isolating stearic from other fatty acids was based on the hypothesis that a mixture of fatty acids heated with a solvent saturated at a given temperature with the acid under determination might be expected on cooling to that temperature to crystallize the whole of the acid sought, provided the other constituents did not increase the solubility. The solvent employed was methylated alcohol (94.4 per cent) saturated with stearic acid at 0.2° C., prepared by chilling a solution of 3 gm. to 1 liter overnight in ice water and siphoning off the saturated mother liquor through a small thistle tube covered with fine calico, using suction. The tests were conducted in a similar manner, taking from 0.5 to 5 gm. of insoluble acids (according to content) to 100 c. c. of alcohol-stearic-acid solution. Shaking was found to increase precipitation. Supersaturation and esterification were recognized as possible sources of error. The method gave concordant results with solid fats containing considerable stearic acid, but slight, if any, precipitate from the acids of butter fat and from mixtures of the acids of Japan wax and pure stearic acid.

Emerson (2) noted considerable variation in the content of different saturated solutions and found that supersaturation seemed to occur when less than 0.7 gm. to 100 c. c. was employed in preparing the solution. The formation of ethyl ester appeared to be a source of error and to have increased the apparent solubility of the stearic acid.

Kreis and Hafner (5) showed that small amounts of stearic acid below 0.1 gm. to 100 c. c. of a saturated solution formed supersaturated solutions, and that less than 0.05 gm. gave low and extremely variable results, even upon the addition of crystals of stearic acid.

Lewkowitsch (6, p. 556–559) claimed that the method yielded capricious results with mixtures of stearic, palmitic, and oleic acids, and that in many cases the results were entirely unreliable when other acids were present. He stated that a considerable proportion of lauric acid would prevent the complete precipitation of stearic acid, even when supersaturated alcohol-stearic-acid solutions were used, and that acids of higher melting point, when present, such as arachic, behenic, etc., would appear in the separated acids. He reported a precipitate of 0.49 per cent from butter fat, of which a portion might be arachic and myristic acids.

The results obtained by various investigators indicate that the solubility of stearic acid increases with the strength of the alcohol, but the figures reported are too variable to warrant further deductions (Table I).

TABLE I.—*Solubility of stearic acid, according to various investigators*

| Investigator. | Approximate strength of alcohol. | Stearic acid to 100 c. c. | Saturation of 100 c. c. at 0° C. |
|---|---|---|---|
| | *Per cent.* | *Gm.* | *Gm.* |
| Hehner and Mitchell (3, p. 323) | 94.4 | 0.2 to 0.5 | 0.1400 to 0.1580 |
| Emerson (2, p. 1754) | 95.5 | .7 | .1223 |
| Do | 95.1 | .7 | .1139 |
| Do | 94.5 | .7 | .1035 |
| Kreis and Hafner (5) | 95 | .5 | .1220 to .1310 |
| Lewkowitsch (6, p. 164) | 94.4 | .3 | .0814 |
| Do | 94.4 | .7 | .0810 to .1082 |
| Ruttan (8, p. 440) | 100 | ..... | .373 |

## PRELIMINARY WORK

In view of what has been stated, the outlook for another investigation was not promising, although Lewkowitsch's final arraignment of the process was not published until nearly a year after the work was undertaken. The subject was of sufficient importance, however, to warrant additional study whatever the outcome.

APPARATUS.—To insure a uniform temperature for crystallization, a tank was constructed of ⅞-inch lumber (20 inches long, 10 inches wide, and 20 inches deep), lined with galvanized iron, provided with a tight cover, and raised by legs to a convenient working height. For icing, a basket (13½ by 6 by 18 inches) of galvanized screening of $\frac{5}{16}$-inch mesh, holding probably 30 pounds of broken ice, was found very satisfactory. The insulation of wood, together with the large volume of water and ice, proved inadequate to meet the requirements of the case, and it was necessary to install in one corner of the tank a pump run by a motor, to keep the water in continuous circulation. With this apparatus a constant temperature of about 0.1° C. was easily maintained (fig. 1, 2).

Several factors had to be considered in the selection of containers in which the tests were to be conducted. They must be of a form, size, and weight suitable for weighing the charge on analytical balances, easily held in position in the tank, and such that the alcoholic solution could be removed while still in the tank, leaving the crystalline residue. After numerous experiments with globe-shaped separatory funnels and filtering tubes, 8-ounce sterilizer bottles were adopted and have been found fairly satisfactory. The bottles are of narrow cylindrical form (2 by 6¾ inches) and are held in place in the tank by pockets of wire screening, with only the rubber stopper and a small portion of the neck projecting out of the water. The solution is siphoned off by means of a small thistle tube (¼-inch bulb) having a felt of absorbent cotton weighing 0.020 gm. supported by a glass bead and covered with a piece of batiste.

REAGENTS.—For the preparation of an alcohol-stearic-acid solution constituents of high quality were deemed essential for satisfactory work. The purification of alcohol had been a subject for study for a number of years in connection with the ordinary analysis of oils and fats, and excellent results were finally secured by treatment with silver nitrate and caustic lime and redistillation. A strength of 95.25 per cent proved a satisfactory solvent for fatty acids, and greater strength was not considered necessary or even advisable.

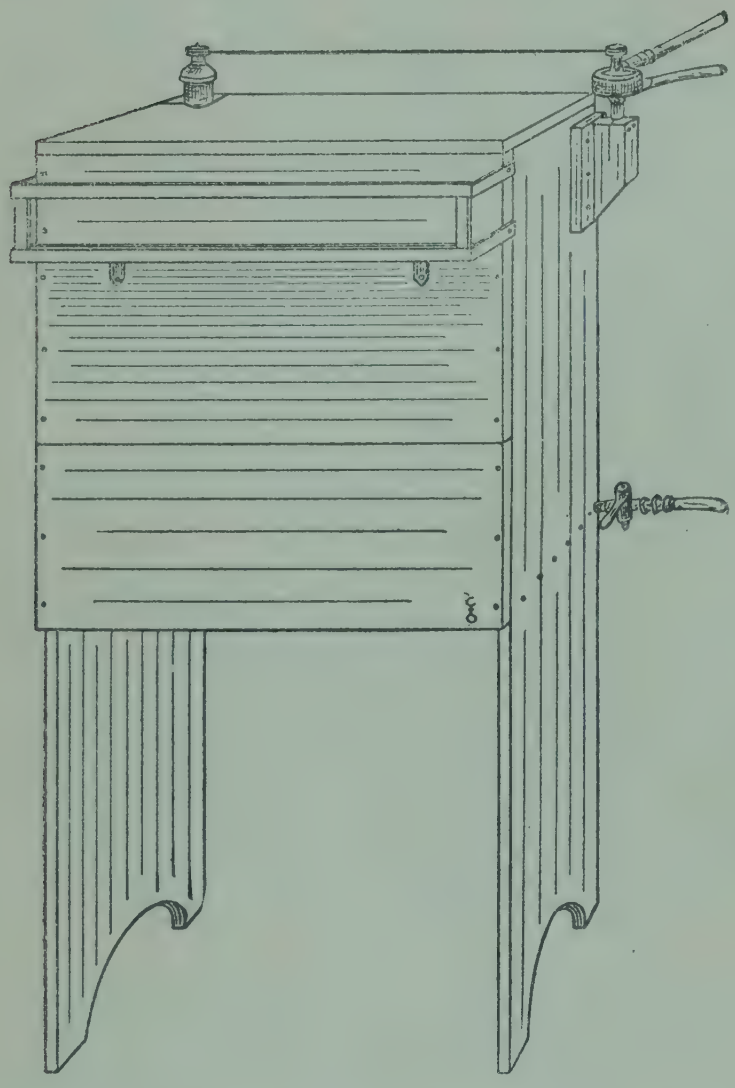

FIG. 1.—Exterior of constant-temperature crystallization tank.

One lot of stearic acid, a mixture of several grades, was purified by fractional distillation of the ethyl ester in vacuo and subsequent repeated crystallization of the separated acids from alcohol as previously described (4). Another lot of acid with a molecular weight of 271.13 was purified by 10 or more crystallizations from alcohol to a molecular weight of 284.25, and a second portion to 284.71, although the resulting leaflets were less perfect than those obtained by the former process.

When using separatory funnels and filtering tubes, alcohol-stearic-acid solutions, saturated at 0.1° C., applied to the insoluble acids of butter at the rate of 150 c. c. to 0.5 gm. of material, seldom yielded an appreciable amount of precipitate on standing, even with the addition of crystals of stearic acid and thorough agitation. Solutions testing about 0.22 and 0.24 gm. of stearic acid to 150 c. c. gave somewhat higher results, although of erratic and untrustworthy character. In the attempt to develop a method with this apparatus, over 140 determinations were made on butter acids, stearic acid, mixtures of butter and stearic acids, stearic and oleic acids, and stearic, myristic, and oleic acids. The object was not attained, and most of the data will be omitted, as

they would serve no useful purpose, merely indicating the time and labor involved. The results, however, with solutions of stearic acid appear to warrant certain deductions.

Solutions containing from 0.25 to 0.29 gm. of stearic acid to 150 c. c. crystallized, leaving a mother liquor of unlike composition (saturation).

The saturation varied inversely with the quantity of stearic acid present.

Presumably, therefore, supersaturation occurred as a result of insufficient stearic acid (Table II).

The time of standing may have had some influence, but when in excess of 24 hours it was of minor consequence.

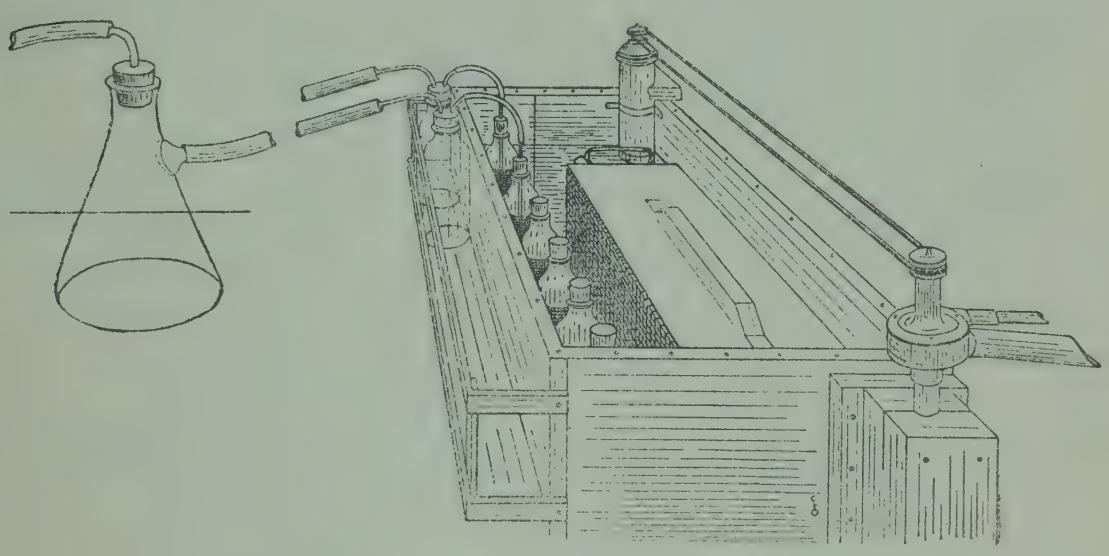

FIG. 2.—Interior of constant-temperature crystallization tank.

The form of the container as viewed in the light of subsequent work was a factor of some importance; a globe-shaped vessel was less effective than a narrow, cylindrical one of large surface.

TABLE II.—*Crystallization of stearic acid from solutions of different content, using separatory funnels*

| Alcohol-stearic-acid solution (grams in 150 c. c.). | Additional stearic acid taken. | Precipitate. | Saturation (grams in 100 c. c.). | Alcohol-stearic-acid solution (grams in 150 c. c.). | Additional stearic acid taken. | Precipitate. | Saturation (grams in 100 c. c.). |
|---|---|---|---|---|---|---|---|
| | *Gm.* | *Gm.* | | | *Gm.* | *Gm.* | |
| 0.2406 | 0.0100 | 0.0130 | 0.1584 | 0.2400 | 0.0304 | 0.0640 | 0.1376 |
| .2406 | .0150 | .0254 | .1535 | .2400 | .0354 | .0733 | .1347 |
| .2406 | .0150 | .0315 | .1494 | .2400 | .0475 | .0872 | .1335 |
| .2406 | .0400 | .0859 | .1298 | .2400 | .0481 | .0910 | .1314 |
| .2406 | .0450 | .0995 | .1241 | .2400 | .0491 | .0910 | .1321 |
| .2400 | .0200 | .0426 | .1449 | .2400 | .0498 | .0960 | .1292 |
| .2400 | .0251 | .0544 | .1405 | | | | |

Stearic-acid solutions were found to crystallize more readily and with greater uniformity in sterilizer bottles than in separatory funnels, probably owing to the more rapid chilling of the narrow column of liquid and more thorough filtration.

Table III shows the amount of stearic acid crystallized from solutions of different content and the saturation of the mother liquor.

TABLE III.—*Crystallization of stearic acid from solutions of different content, using sterilizer bottles*

| Alcohol. | Stearic acid taken. | Precipitate. | Saturation (grams in 100 c. c.). | Alcohol. | Stearic acid taken. | Precipitate. | Saturation (grams in 100 c. c.). |
|---|---|---|---|---|---|---|---|
| *C. c.* | *Gm.* | *Gm.* | | *C. c.* | *Gm.* | *Gm.* | |
| 150 | 0. 2000 | 0. 0000 | ..... | 150 | 0. 3670 | 0. 1880 | 0. 1193 |
| 150 | . 2400 | . 0020 | 0. 1587 | 150 | . 3800 | . 2000 | . 1200 |
| 150 | . 2705 | . 0485 | . 1480 | 150 | . 4000 | . 2210 | . 1193 |
| 150 | . 2815 | . 0700 | . 1410 | 150 | . 4080 | . 2260 | . 1213 |
| 150 | . 3055 | . 1110 | . 1297 | 150 | . 4200 | . 2435 | . 1177 |
| 150 | . 3215 | . 1280 | . 1290 | 150 | . 4650 | . 2980 | . 1113 |
| 150 | . 3475 | . 1680 | . 1197 | 150 | . 5000 | . 3255 | . 1163 |
| 150 | . 3600 | . 1815 | . 1190 | 150 | . 6000 | . 4315 | . 1123 |

TABLE IV.—*Crystallization of stearic acid from solutions of different content, using sterilizer bottles*

| Alcohol-stearic-acid solution (0.3990 gm. in 150 c. c.). | Alcohol. | Equivalent in stearic acid (grams in 150 c. c.). | Precipitate. | Saturation (grams in 100 c. c.). |
|---|---|---|---|---|
| | *C. c.* | | *Gm.* | |
| 100 | 50 | 0. 2660 | 0. 0555 | 0. 1403 |
| 110 | 40 | . 2926 | . 0980 | . 1297 |
| 120 | 30 | . 3192 | . 1500 | . 1128 |
| 130 | 20 | . 3458 | . 1745 | . 1142 |
| 140 | 10 | . 3724 | . 2055 | . 1113 |
| 150 | 0 | . 3990 | . 2335 | . 1103 |

## APPLICATION OF CRYSTALLIZATION METHOD

The facility with which alcohol-stearic-acid solutions crystallize increased with the concentration. Solutions of 0.40 to 0.45 gm. to 150 c. c. formed crystals readily, gave a satisfactory amount of precipitate, and when applied to the insoluble acids of butter yielded an additional amount from that source. This would indicate that if the stearic-acid content of the solution is sufficient, crystallization of stearic from butter acids is no more difficult than from other products. The results were very concordant for a crystallization method when all details of manipulation were strictly observed: The water maintained at the required level, properly iced at all times, and the pump run continuously at good speed. A gentle agitation of the solution after standing overnight in the ice tank assisted in completing the precipitation, but anything in

the nature of shaking reduced the fragile crystals to a mass and rendered filtration extremely difficult or impossible.

## EXPERIMENTAL METHOD IN DETAIL

Five-tenths of a gram of melted insoluble acids are placed in an 8-ounce sterilizer bottle and 150 c. c. of an alcohol-stearic-acid solution (3 gm. to 1,000 c. c.), accurately measured with a pipette at 30° C., added. The bottle is sealed with a solid-rubber stopper, shaken at a gradually increasing temperature until a clear solution is obtained, placed immediately in a pocket of the ice tank, and allowed to stand overnight. The following morning the solution is gently agitated by inverting the bottle several times, and in the afternoon it is siphoned off as thoroughly as possible by means of a small thistle tube and a perforated rubber stopper, using suction. The residue is dissolved in ethyl ether, transferred to a tared 140 c. c. wide-mouth Erlenmeyer flask, the ether carefully distilled off, the residue dried at 100° C., and weighed. As saturation may vary somewhat with the amount of stearic acid present and as the quantity of solution retained by the precipitate depends in a measure on the amount of precipitate, blanks are run on a weight of stearic acid equivalent to that expected in the test. By deducting the additional stearic acid taken from the weight recovered the true blank for the alcohol-stearic-acid solution is obtained.

## NATURE OF THE PRECIPITATE

To ascertain whether the crystalline substance obtained from butter acids was stearic acid or a mixture, the residues from a number of tests (one being insufficient for accurate work) were combined and the molecular weight determined by saponification. Such a determination made after securing satisfactory control of the stearic-acid method gave 284.64, theoretically 284.288. The melting point was not determined, as it was considered less reliable than the molecular weight.

## INFLUENCE OF DIFFERENT FATTY ACIDS ON PRECIPITATION OF STEARIC ACID

Numerous tests were made in an effort to determine whether lauric, myristic, palmitic, and oleic acids had any effect on the crystallization of stearic acid and, if so, the nature and extent of such action. Table V will serve to illustrate.

According to molecular-weight determinations the lauric and palmitic acids were of excellent quality and the myristic and oleic acids somewhat inferior.

Lauric, myristic, and oleic acids in relatively large amounts showed no appreciable influence on the crystallization of stearic acid. Palmitic acid, on the other hand, noticeably increased the solubility and affected the crystalline structure of the precipitate.

TABLE V.—*Effect of different fatty acids on precipitation of stearic acid*

| Alcohol-stearic acid solution (grams in 150 c. c.). | Additional stearic acid taken. | Other acids taken. | Precipitate. | Saturation (grams in 100 c. c.). |
|---|---|---|---|---|
| | *Gm.* | *Gm.* | *Gm.* | |
| **STEARIC ACID** | | | | |
| 0.3990 | 0.1000 | ..... | 0.3420 | 0.1047 |
| .3990 | .1015 | ..... | .3430 | .1050 |
| .3990 | .1035 | ..... | .3415 | .1073 |
| .3990 | .1000 | ..... | .3405 | .1057 |
| **LAURIC ACID** | | | | |
| 0.3990 | .1030 | 0.4000 | .3455 | .1043 |
| .3990 | .1000 | .4000 | .3415 | .1050 |
| .3990 | .1000 | .4000 | .3430 | .1040 |
| .3990 | .1010 | .4000 | .3450 | .1033 |
| **MYRISTIC ACID** | | | | |
| 0.3990 | .1000 | .4000 | .3495 | .0997 |
| .3990 | .1010 | .4000 | .3480 | .1013 |
| .3990 | .1000 | .4000 | .3490 | .1000 |
| .3990 | .1000 | .4000 | .3515 | .0983 |
| **PALMITIC ACID** | | | | |
| 0.3990 | .1055 | .4000 | .3135 | .1273 |
| .3990 | .1000 | .4030 | .2980 | .1340 |
| .3990 | .1010 | .2500 | .2965 | .1357 |
| .3990 | .1040 | .2500 | .3065 | .1310 |
| .3990 | .1050 | .2000 | .3085 | .1303 |
| **OLEIC ACID** | | | | |
| 0.3990 | .1070 | .4220 | .3515 | .1030 |
| .3990 | .1010 | .4255 | .3440 | .1040 |
| .3990 | .1000 | .4000 | .3485 | .1003 |
| .3990 | .1035 | .4000 | .3460 | .1043 |

The addition of palmitic acid to butter acids reduced the amount of stearic acid recovered in the test. Some of our more recent determinations indicated that the solvent action of palmitic acid can be counteracted in a large measure, if not entirely, by increasing the relative amount of stearic acid in solution. With butter acids of average palmitic acid content, an alcohol-stearic-acid solution, containing at least 3 gm. of stearic acid to the liter, is necessary and possibly 3.4 or 3.7 gm. may prove more reliable. This, however, seems to depend to a considerable degree upon the alcohol-stearic-acid solution employed. Some solutions made

from purified alcohol of approximately the same strength require more stearic acid than others to insure a constant saturation, the reason for which we have been unable as yet to determine. Some of the results cited in Tables VI to VIII are probably low, owing to insufficient stearic acid in solution, although the results are all calculated with reference to blank tests conducted under precisely like conditions.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*

| Sample No. | Insoluble acids of butter taken. | Alcohol-stearic-acid solution (grams in 150 c. c.). | Additional stearic acid taken. | Precipitate. | Blank. | Saturation (grams in 100 c. c.). | Stearic acid. |
|---|---|---|---|---|---|---|---|
| | *Gm.* | | *Gm.* | *Gm.* | *Gm.* | | *Per cent.* |
| Solution A. (0.8153)[a] | ..... | 0.3990 | 0.0525 | 0.2900 | 0.2375 | 0.1077 | ..... |
| | ..... | .3990 | .0500 | .2880 | .2380 | .1073 | ..... |
| | ..... | .3990 | .0500 | .2895 | .2395 | .1063 | ..... |
| | | | | | [b] .2383 | | |
| Solution B. (0.8135)[a] | ..... | .3960 | .0515 | .2630 | .2115 | .1230 | ..... |
| | ..... | .3960 | .0530 | .2640 | .2110 | .1233 | ..... |
| | ..... | .3960 | .0505 | .2625 | .2120 | .1227 | ..... |
| | ..... | .3960 | .0490 | .2605 | .2115 | .1230 | ..... |
| | ..... | .3960 | .0500 | .2610 | .2110 | .1233 | ..... |
| | | | | | [b] .2114 | | |
| Solution C. (0.8142)[a] | ..... | .4050 | .0575 | .2770 | .2195 | .1237 | ..... |
| | ..... | .4050 | .0500 | .2710 | .2210 | .1227 | ..... |
| | ..... | .4050 | .0800 | .2990 | .2190 | .1240 | ..... |
| | ..... | .4050 | .0800 | .3005 | .2205 | .1230 | ..... |
| | | | | | [b] .2200 | | |
| Solution D. (0.8142)[a] | ..... | .4050 | .0820 | .3040 | .2220 | .1220 | ..... |
| | ..... | .4050 | .0805 | .3045 | .2240 | .1207 | ..... |
| | | | | | [b] .2230 | | |
| Solution E. (0.8147)[a] | ..... | .4470 | .1115 | .3765 | .2650 | .1213 | ..... |
| | ..... | .4470 | .1115 | .3765 | .2650 | .1213 | ..... |
| | | | | | [b] .2650 | | |
| Solution F. (0.8147)[a] | ..... | .4440 | .1105 | .3840 | .2735 | .1137 | ..... |
| | ..... | .4440 | .1100 | .3830 | .2730 | .1140 | ..... |
| | | | | | [b] .2733 | | |
| Solution A: | | | | | | | |
| 4 ..... | .5440 | .3990 | ..... | .2930 | .2383 | ..... | 10.96 |
| 4 ..... | .5170 | .3990 | ..... | .2900 | .2383 | ..... | 10.00 |
| 4 ..... | .5235 | .3990 | ..... | .2915 | .2383 | ..... | 10.16 |
| 4 ..... | .5000 | .3990 | ..... | .2905 | .2383 | ..... | 10.44 |
| | | | | | | | [b] 10.17 |
| Solution B: | | | | | | | |
| 5 ..... | .5085 | .3960 | ..... | .2460 | .2114 | ..... | 6.80 |
| 5 ..... | .5190 | .3960 | ..... | .2480 | .2114 | ..... | 7.05 |
| | | | | | | | [b] 6.93 |
| 6 ..... | .5230 | .3960 | ..... | .2555 | .2114 | ..... | 8.43 |
| 6 ..... | .5010 | .3960 | ..... | .2520 | .2114 | ..... | 8.10 |
| | | | | | | | [b] 8.27 |
| 7 ..... | .5135 | .3960 | ..... | .2515 | .2114 | ..... | 7.81 |
| 7 ..... | .5230 | .3960 | ..... | .2500 | .2114 | ..... | 7.38 |
| | | | | | | | [b] 7.60 |

[a] Hydrometer reading at 15.50° C. of the alcohol employed. [b] Average.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*—Continued

| Sample No. | Insoluble acids of butter taken. | Alcohol-stearic-acid solution (grams in 150 c. c.). | Additional stearic acid taken. | Precipitate. | Blank. | Saturation (grams in 100 c. c.). | Stearic acid. |
|---|---|---|---|---|---|---|---|
| Solution A: | *Gm.* | | *Gm.* | *Gm.* | *Gm.* | | *Per cent.* |
| 8 | 0.5170 | 0.3990 | | 0.2850 | 0.2383 | | 9.03 |
| 8 | .5120 | .3990 | | .2840 | .2383 | | 8.93 |
| 8 | .5225 | .3990 | | .2830 | .2383 | | 8.56 |
| | | | | | | | [a] 8.84 |
| Solution C: | | | | | | | |
| 9 [b] | .5090 | .4050 | | .2970 | .2200 | | 15.13 |
| 9 | .5155 | .4050 | | .3000 | .2200 | | 15.52 |
| 9 | .5150 | .4050 | | .2970 | .2200 | | 14.95 |
| 9 | .5130 | .4050 | | .2980 | .2200 | | 15.20 |
| | | | | | | | [a] 15.20 |
| Solution B: | | | | | | | |
| 10 [b] | .5130 | .3960 | | .3000 | .2114 | | 17.27 |
| 10 | .5050 | .3960 | | .3020 | .2114 | | 17.94 |
| Solution C: | | | | | | | |
| 10 [b] | .4995 | .4050 | | .3070 | .2200 | | 17.42 |
| 10 | .5060 | .4050 | | .3075 | .2200 | | 17.29 |
| 10 | .5255 | .4050 | | .3140 | .2200 | | 17.89 |
| | | | | | | | [a] 17.56 |
| Solution B: | | | | | | | |
| 11 [b] | .5065 | .3960 | | .2990 | .2114 | | 17.30 |
| 11 | .5150 | .3960 | | .3000 | .2114 | | 17.20 |
| | | | | | | | [a] 17.25 |
| Solution C: | | | | | | | |
| 14 | .5165 | .4050 | | .2645 | .2200 | | 8.62 |
| 14 | .5070 | .4050 | | .2635 | .2200 | | 8.58 |
| 14 | .5015 | .4050 | | .2625 | .2200 | | 8.47 |
| | | | | | | | [a] 8.56 |
| 15 | .5045 | .4050 | | .2705 | .2200 | | 10.01 |
| 15 | .5060 | .4050 | | .2715 | .2200 | | 10.18 |
| | | | | | | | [a] 10.10 |
| 16 | .5265 | .4050 | | .2665 | .2200 | | 8.83 |
| 16 | .5205 | .4050 | | .2680 | .2200 | | 9.22 |
| | | | | | | | [a] 9.03 |
| 17 [c] | .5045 | .4050 | | .2965 | .2200 | | 15.16 |
| 17 | .5300 | .4050 | | .2980 | .2200 | | 14.72 |
| | | | | | | | [a] 14.94 |
| 18 [c] | .5035 | .4050 | | .2945 | .2200 | | 14.80 |
| 18 | .4990 | .4050 | | .2930 | .2200 | | 14.63 |
| 18 | .5105 | .4050 | | .2940 | .2200 | | 14.50 |
| | | | | | | | [a] 14.64 |
| 19 [c] | .5205 | .4050 | | .2925 | .2200 | | 13.93 |
| 19 | .5110 | .4050 | | .2905 | .2200 | | 13.80 |
| | | | | | | | [a] 13.87 |
| Solution D: | | | | | | | |
| 20 | .5180 | .4050 | | .2720 | .2230 | | 9.46 |
| 20 | .5025 | .4050 | | .2715 | .2230 | | 9.65 |
| | | | | | | | [a] 9.56 |
| 21 | .5090 | .4050 | | .2630 | .2230 | | 7.86 |
| 21 | .5205 | .4050 | | .2650 | .2230 | | 8.07 |
| | | | | | | | [a] 7.97 |

[a] Average. [b] The cows were fed beef tallow. [c] The cows were fed palm oil.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*—Continued

| Sample No. | Insoluble acids of butter taken. | Alcohol-stearic-acid solution (grams in 150 c. c.). | Additional stearic acid taken. | Precipitate. | Blank. | Saturation (grams in 100 c. c.). | Stearic acid. |
|---|---|---|---|---|---|---|---|
| Solution F: | *Gm.* | | *Gm.* | *Gm.* | *Gm.* | | *Per cent.* |
| I | 0.5070 | 0.4440 | ..... | [a]0.3850 | 0.2733 | ..... | 22.03 |
| I | .5225 | .4440 | ..... | .3915 | .2733 | ..... | 22.62 |
| | | | | | | | [b]22.33 |
| II | .5205 | .4440 | ..... | [a].3830 | .2733 | ..... | 21.08 |
| II | .5215 | .4440 | ..... | .3850 | .2733 | ..... | 21.42 |
| | | | | | | | [b]21.25 |
| Solution E: | | | | | | | |
| III | .5090 | .4470 | ..... | [c].3525 | .2650 | ..... | 17.19 |
| III | .5140 | .4470 | ..... | .3525 | .2650 | ..... | 17.02 |
| III | .5060 | .4470 | ..... | .3535 | .2650 | ..... | 17.49 |
| | | | | | | | [b]17.23 |
| IV | .5015 | .4470 | ..... | [c].3505 | .2650 | ..... | 17.05 |
| IV | .5035 | .4470 | ..... | .3520 | .2650 | ..... | 17.28 |
| | | | | | | | [b]17.17 |

[a] Molecular weight of the several precipitates, 284.54.
[b] Average.
[c] Molecular weight of the several precipitates, 284.59.

TABLE VII.—*Amount of stearic acid in the insoluble acids of beef tallow*

| Sample No. | Insoluble acids of beef tallow taken. | Alcohol-stearic-acid solution (grams in 150 c. c.). | Additional stearic acid taken. | Precipitate. | Blank. | Saturation (grams in 100 c. c.). | Stearic acid. |
|---|---|---|---|---|---|---|---|
| | *Gm.* | | *Gm.* | *Gm.* | *Gm.* | | *Per cent.* |
| Solution A | ..... | 0.3990 | 0.1500 | 0.3870 | 0.2370 | 0.1080 | ..... |
| Do | ..... | .3990 | .1555 | .3930 | .2375 | .1077 | ..... |
| | | | | | [a].2373 | | |
| Solution B | ..... | .3960 | .1520 | .3690 | .2170 | .1193 | ..... |
| Do | ..... | .3960 | .1550 | .3700 | .2150 | .1207 | ..... |
| | | | | | [a].2160 | | |
| Solution A: | | | | | | | |
| 1 | 0.5280 | .3990 | ..... | .3975 | .2373 | ..... | 30.34 |
| 1 | .5155 | .3990 | ..... | .3960 | .2373 | ..... | 30.79 |
| | | | | | | | [a]30.57 |
| Solution B: | | | | | | | |
| 2 | .5025 | .3960 | ..... | .3740 | .2160 | ..... | 31.44 |
| 2 | .5150 | .3960 | ..... | .3775 | .2160 | ..... | 31.36 |
| | | | | | | | [a]31.40 |

[a] Average.

TABLE VIII.—*Amount of stearic acid in the insoluble acids of palm oil*

| Sample No. | Insoluble acids of palm oil taken. | Alcohol-stearic-acid solution (grams in 150 c. c.). | Additional stearic acid taken. | Precipitate. | Blank. | Saturation (grams in 100 c. c.). | Stearic acid. |
|---|---|---|---|---|---|---|---|
| | *Gm.* | | *Gm.* | *Gm.* | *Gm.* | | *Per cent.* |
| Solution C | ..... | 0.4050 | 0.1515 | 0.3745 | 0.2230 | 0.1213 | ..... |
| Do | ..... | .4050 | .1500 | .3750 | .2250 | .1200 | ..... |
| Do | ..... | .4050 | .2000 | .4255 | .2255 | .1197 | ..... |
| Do | ..... | .4050 | .2030 | .4295 | .2265 | .1190 | ..... |
| | | | | | [a].2250 | | |
| Solution C: | | | | | | | |
| 12 | 0.3405 | .4050 | .1500 | [b].4040 | .2250 | ..... | 8.52 |
| 12 | .4110 | .4050 | .1510 | .4100 | .2250 | ..... | 8.27 |
| 12 | .5205 | .4050 | .1540 | .4265 | .2250 | ..... | 9.13 |
| 12 | .5000 | .4050 | .1500 | .4205 | .2250 | ..... | 9.10 |
| 12 | .5215 | .4050 | .1500 | .4245 | .2250 | ..... | 9.49 |
| 12 | .5135 | .4050 | .1565 | .4275 | .2250 | ..... | 8.96 |
| | | | | | | | [a]8.91 |

[a] Average. [b] Molecular weight of the several precipitates, 284.38.

The stearic acid obtained from the insoluble acids of butter fat by the method described ranges from 7 to 22 per cent, which is considerably in excess of the amount generally credited to the product. The prevailing opinion was supported undoubtedly by the fact that only a small amount of precipitate is obtainable by the Hehner and Mitchell (3) method, as shown by several investigators.

The amount of stearic acid appears to be affected by the feed the animal receives. Samples 9, 10, and 11, averaging 16.67 per cent, were from cows fed beef tallow; samples 17, 18, and 19, averaging 14.48 per cent, were from those fed palm oil; while samples 4 to 8, 14 to 16, 20 and 21, averaging 8.70 per cent, were from those fed a ration low in fat. It is probable that the individuality of the animal and period of lactation also affect the composition. The entire matter of the effect of food as well as other influences upon the chemical character of butter fat is now being further studied.

The stearic acid (8.91 per cent) recovered from the insoluble acids of palm oil exceeded the amount usually reported.

## SUMMARY

The results of the determinations of stearic acid in the insoluble acids of butter fat by the method proposed show a higher percentage of stearic acid than has been generally reported. The facts that the results are concordant and that the molecular weight determinations of the crystallized product secured by the proposed method agree closely with the theoretical molecular weight leave no doubt as to the identity and approximate purity of the stearic acid.

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# LIFE HISTORY AND HABITS OF TWO NEW NEMATODES PARASITIC ON INSECTS[1]

[PRELIMINARY PAPER]

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## INTRODUCTION

While investigating the life history and methods of control of the elm borer (*Saperda tridentata* Oliv.) and the termite (*Leucotermes lucifugus* Rossi) at the Kansas Agricultural Experiment Station, two new nematodes were found, one parasitic on the former and the other parasitic on the latter. One hundred and twenty-one adult beetles obtained from one tree[2] were placed in breeding cages, but in no instance were eggs deposited, and both sexes eventually weakened and died. Examination after death showed that the intestines were so filled with nematodes that in only one female were eggs even developed in the body. The death rate due to nematode parasitization was apparently 100 per cent. Several colonies of *Leucotermes lucifugus* were placed in salve boxes, together with food. Inasmuch as *Saperda tridentata* had shown so high a nematode parasitization, it was naturally suggested that nematodes might be present in the termites. Accordingly a number of these insects were killed and examined, with the result that nematodes were found infesting the head in varying degrees. Of the colonies taken, 76.92 per cent were parasitized with nematodes. The parasitism of the individuals in single colonies ranged from 0 to 100 per cent.

## DIPLOGASTER LABIATA

The nematodes were submitted to Dr. N. A. Cobb, of the Bureau of Plant Industry, United States Department of Agriculture, for identification. He found that the nematode parasitizing *Saperda tridentata* was a new species which he named "*Diplogaster labiata*" (fig. 1; 2, *A–H*), and described as follows:

**Diplogaster labiata,** n. sp. $\frac{1.2\ \ 17.\ \ 21.\ \ '59'^{48}\ \ 91.}{2.1\ \ 4.2\ \ 4.2\ \ 4.4\ \ 2.9}$ 0.66 mm. (The formula was derived from a single specimen.) The thin layers of the transparent, colorless, naked cuticle are traversed by fine transverse striæ, resolvable with high powers into rows of dots, more particularly near the head and on the tail, those on the tail being somewhat irregularly placed. The cuticle is also longitudinally striated, and the dots of the transverse striations are coincident with those of the longitudinal striations. The longi-

[1] Contribution from the Entomological Laboratory, Kansas State Agricultural College, No. 17. This paper embodies the results of some of the investigations undertaken by the authors in the prosecution of projects Nos. 13 and 101, Kansas Agricultural Experiment Station.

[2] A tent was placed around an elm tree so that all emerging insects might be secured for breeding purposes.

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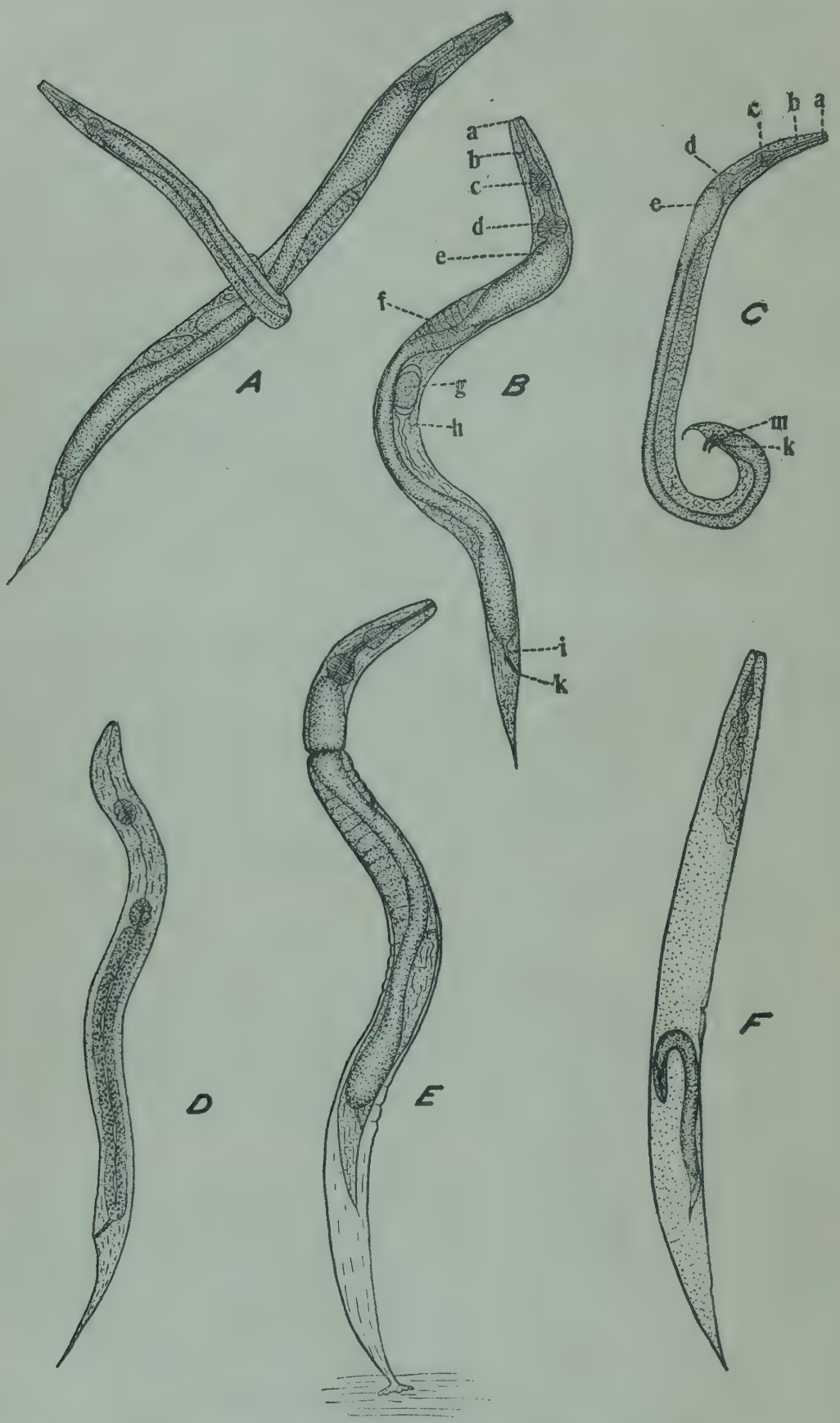


FIG. 1.—*Diplogaster labiata: A*, Mating (× 125); *B*, mature female reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *f*, ovaries, *g*, egg, *h*, genital pore, *i*, rectum, *k*, anus; *C*, mature male reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *k*, anus, *m*, spicula; *D*, at time of hatching (× 400); *E*, female during process of molting (× 125); *F*, dead female with young nematode which hatched within her body (× 125). Drawings by A. L. Ford.

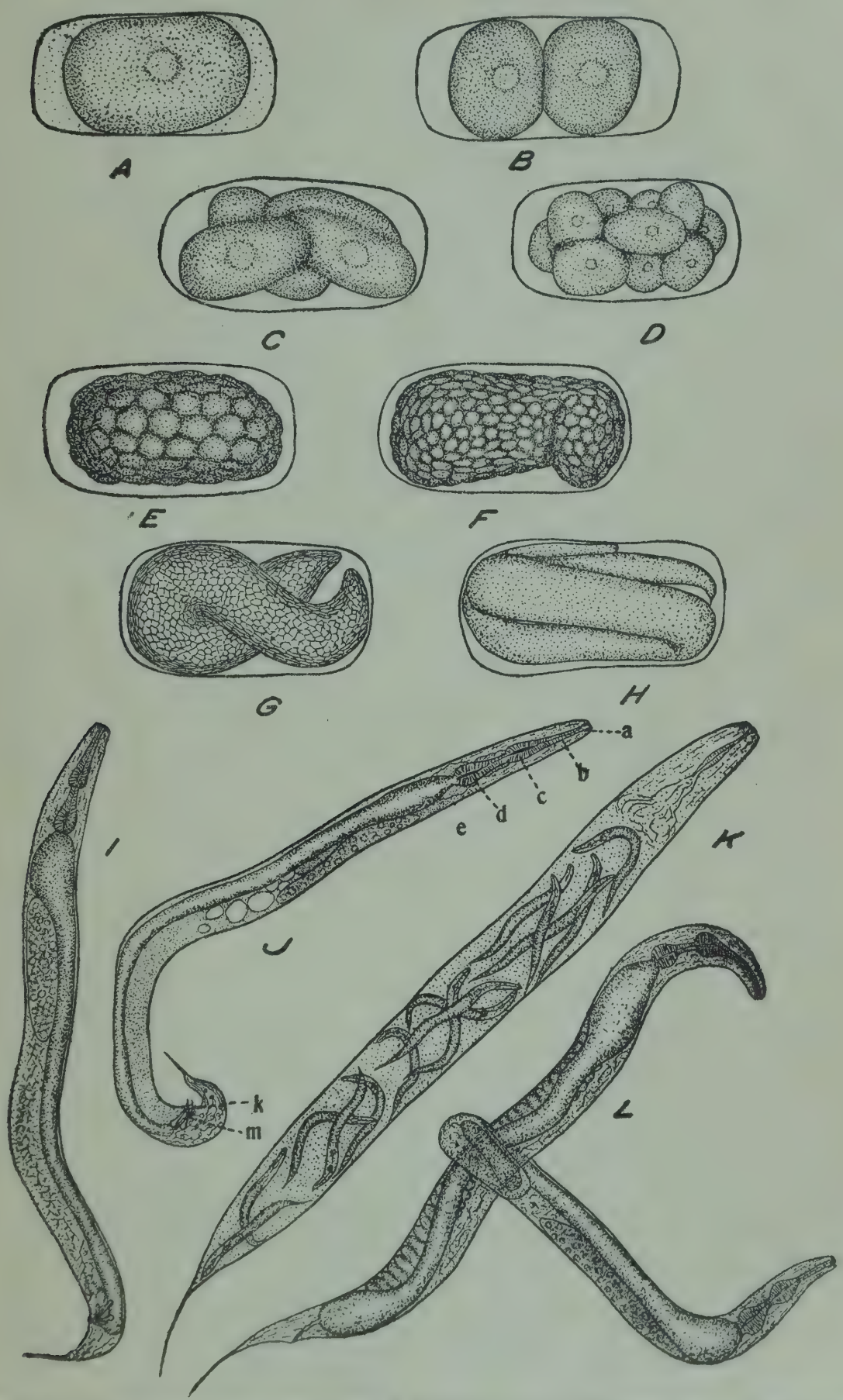


FIG. 2.—*A–H, Diplogaster labiata:* Development of the egg (× 500); *I*, Diplogaster aerivora: mature male reared in moist soil (× 160); *J, Diplogaster aerivora:* mature male reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *k*, anus, *m*, spicula; *K, Diplogaster aerivora:* dead female with young which hatched within her body (× 125); *L, Diplogaster aerivora:* mating (× 125). Drawings by A. L. Ford.

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tudinal striæ are not present on the lateral fields, this naked space being one-third to one-half the width of the body. The slightly conoid neck becomes slightly convex-conoid near the head, the lip region of which is set off by a very broad, almost imperceptible constriction. There are six strongly developed and fairly distinct lips, each ending in a conoid tip, from the summit of which issues a very short innervated bristle-like papilla. The lips have a more or less distinct refractive framework and are in all probability quite mobile. Usually in specimens which have been fixed in Flemming's solution the tips of the lips are slightly outward-pointing, leaving a somewhat circular refractive mouth opening about two-fifths as wide as the front of the head. The inner surface of the lips is so strongly refractive that usually the posterior limits of the lips are distinctly visible, more particularly as the wall of the pharynx at this point is encircled by a very delicate refractive line lying considerably in front of the middle of the pharynx. This latter appears to be irregularly cylindroid, but is slightly unsymmetrical at the base. On the whole, it is about two-fifths as wide as the head. It appears to possess at the base a rather well-developed but blunt, slightly inward-projecting process or tooth. In the lateral view, as the posterior part of the pharynx appears to pass around this projection, it acquires the slightly unsymmetrical contour already mentioned. The walls of the esophagus are rather distinctly ceratinized. The esophagus begins at the base of the pharynx as a tube two-thirds as wide as the base of the head and continues to have this diameter, or a slightly greater, until it reaches a point halfway back to the median bulb. Thence onward it diminishes slightly, so that just in front of the median bulb it is only half as wide as the middle of the neck. The median bulb is a well-developed, elongated or ellipsoidal, radially muscular structure, with a somewhat distinct elongated but narrow valve. This bulb is about two-thirds as wide as the middle of the neck. Behind the median bulb the esophageal tube continues with a diameter one-third to two-fifths as great as the corresponding portion of the neck but diminishes very slightly, so that just in front of the ellipsoidal cardiac bulb it is less than one-third as wide as the corresponding portion of the neck. The cardiac bulb contains a rather distinct and rather complicated threefold valvular apparatus and is capable of opening out posteriorly, so that the lumen of the posterior part of the bulb, where it debouches into the intestine, then becomes one-fourth as wide as the corresponding portion of the body. The lining of the esophagus is a distinct feature throughout its length. The intestine, which is thin-walled at first, is separated from the esophagus by a distinct constriction. It becomes at once four-fifths to five-sixths as wide as the body and presents at the beginning a distinct cardiac cavity. There is also a distinct cardia. The cells of the intestine, which are of such size that probably four are required to build a circumference, contain rather large nuclei and are packed with granules of variable size, the largest of which have a diameter as great as the distance between two of the longitudinal striæ, the smallest of which are very much smaller. The lining of the intestine is refractive, so that the lumen is usually quite a distinct feature. From the slightly raised anus the narrow, refractive, ceratinized rectum, which is one and one-half to two times as long as the anal body diameter, extends inward and forward. The tail end begins to taper from some distance in front of the anus but in front of the anus tapers only very slightly. Behind the anus it tapers rather regularly to an acute point. Near the middle of the tail there appears to be a lateral papilla on each side. From the slightly raised, rather broad vulva the vagina leads inward at right angles to the ventral surface nearly halfway across the body, where it joins the two uteri, which extend in opposite directions. The reflexed ovaries reach more than halfway back to the vulva, at any rate in apparently young specimens in which no eggs exist in the uterus. The ova in the ovary are arranged more or less single file for about half its length; toward the blind end they are arranged irregularly. Fertilized females show sperm cells in the uterus of such a

size that about four to five side by side would span the body diameter. Numerous micro-organisms were seen in the intestine.

Male formula. $\frac{1.9 \quad 16. \quad 21. \quad M'^{59.} \quad 94.}{1.7 \quad 3.1 \quad 3.5 \quad 3.9 \quad 2.9}$ 0.72 mm. (single specimen). The tail of the male differs materially in form from that of the female. It begins to taper at the anus, and it tapers rapidly in the anterior two-thirds, more particularly in the middle third, so that at the beginning of the final third it is only about one-tenth as wide as at the anus. Thence onward it tapers rather regularly to the exceedingly fine terminus; there is, however, a pronounced ventral elevation at the beginning of the small part of the tail, though it remains uncertain whether this elevation is innervated. The middle portion of the tail is strongly convex-conoid, the convexity existing largely on the dorsal side. The cuticle of the tail presents a peculiar arrangement of the dots, such that there is an appearance of two sets of oblique fibers crossing each other, these fibers being arranged approximately at 45° to the longitudinal lines. The two equal, rather uniform, somewhat arcuate, blunt spicula are about one and one-fourth to one and one-half times as long as the anal body diameter. Their proximal ends, which are slightly narrower than the main portion, are set off by a rather broad and prominent constriction. At their widest part, through the middle, they are about one-fifth to one-sixth as wide as the corresponding portion of the body. The accessory piece is about half as long as the spicula. It is very inconspicuous near the anus, but lies parallel to the spicula. It widens out to a somewhat clavate or elongated pyriform contour, and has its rounded proximal end toward the dorsal side of the body, and from this blunt end muscular fibers pass obliquely backward to the ventral surface of the tail and join the caudal wall at a distance nearly half way from the anus to the beginning of the narrow portion. Oblique copulatory muscles are to be seen opposite the ejaculatory duct for a distance about one and one-half times as great as the length of the tail. The male papillæ are arranged as follows: One ventrally submedian pair a little in front of the proximal ends of the spicula; one ventrally submedian pair a little in front of the anus, and one ventrally sublateral pair on the same zone; another sublateral pair just opposite the anus; a lateral pair slightly behind the middle of the enlarged portion of the tail; a submedian pair nearly halfway from that last mentioned to the beginning of the small part of the tail; a dorsally sublateral pair a little in front of the beginning of the narrow portion of the tail; three subventral pairs close together opposite that last mentioned; between the members of these three subventral pairs, possibly a single ventral papilla. The most pronounced of these papillæ can hardly be called digitate. The ejaculatory duct is about two-fifths as wide as the body. The vas deferens is nearly two-thirds as wide as the body. The testis tapers so that at the point of inflection, a short distance behind the cardiac bulb, it is about one-fourth as wide as the body. The blind end lies about two body widths behind the flexure.

Habitat: Manhattan, Kans., 1915, on *Saperda tridentata*.

The eggs of *Diplogaster labiata*, elliptical in shape, about twice as long as wide, with bluntly rounded ends, when freshly deposited, were uniformly dark brown or gray, but after segmentation began they became darker. Their average length was 0.0627 mm. and the average diameter 0.031 mm. They were laid singly with apparently no preference as to the place of deposition. Occasionally segmentation began before the eggs were deposited. From the beginning of segmentation the cell divisions could be plainly followed throughout (fig. 2, *A–H*).

A few hours before emerging, the folded young nematodes made slight movements within the egg. Later these movements became

more vigorous until finally they ruptured the shells and emerged, after which the egg walls collapsed. Occasionally a young nematode hatched within the body of a dead female. In cultures the eggs hatched in from 30 to 32 hours from the time of deposition, and the nematodes matured in from 7 to 10 days. The males appeared to mature slightly in advance of the females.

At hatching, the young nematodes were about 0.2 mm. in length (fig. 1, *D*), very slender, and sluggish, and remained for a time in a curled position. Later they straightened out their bodies and became very active. The young worms were almost transparent (in water cultures), there being no solid food in the alimentary canal. As development proceeded, the young became darker in color and more active. At the end of 5 days the sex organs began to appear, and in from 7 to 10 days the nematodes reached maturity.

Specimens which were isolated and kept under observation were noted to molt at least three times, these molts occurring about three days apart. The process of molting (fig. 1, *E*) was as follows: The nematode first fastened its posterior end to any surface upon which it might be resting. The skin then broke at the anterior end and the nematode began to emerge. At first the process was very slow, owing to the fact that the opening of the molt skin was smaller in diameter than the middle part of the body. By moving vigorously from side to side, the nematode slowly worked its way out of the skin. After the widest portion of the body had passed through the opening, no further resistance to emergence was offered, as the posterior end rapidly decreased in diameter. The nematodes were not always able to emerge, as occasionally specimens were found which died before completing the process. Molting lasted from 45 minutes to 6 hours.

The adults and the young were similar in form and food habits, but differed in that the adults possessed sex organs. The mature females were about 0.7 mm. in length and 0.03 mm. in diameter, while the males were about 0.6 mm. in length and 0.02 mm. in diameter.

As soon as maturity was reached, mating began (fig. 1, *A*). The male fastened its caudal end around the middle of the female's body. During this process the male held its body rigid, while the female moved vigorously from side to side. It was not uncommon to find males in the act of mating with their bodies wrapped twice about the females. Toward the end of the process the female increased her activity and soon shook the male free. Many matings were observed, the shortest of which lasted about 2 minutes and the longest 30 minutes.

PROPORTION OF SEXES.—Of 367 specimens examined, 229 were found to be females and 138 were males. In other cultures in which counts were not made the females were noticed to be more abundant than the males.

PERIOD OF OVIPOSITION.—While in the specimens of *Diplogaster labiata* under observation mating usually occurred but once, occasionally a few individuals mated a second time. Oviposition began from two to four hours after mating and lasted over a period of about two days, during which time the average number of eggs deposited was seven.

HABITS.—These nematodes infested the intestines of adults of *Saperda tridentata* in such large numbers that they prevented these insects from performing their natural functions. They lived in the alimentary canal in such large numbers that they ruptured the walls of the canal and, escaping into the body cavity of the insect, caused its death.

The examination of individuals of *Saperda tridentata* which had died in this manner rarely showed eggs that had started to develop. Specimens of *Diplogaster labiata* placed in water cultures were fed on macerated bodies of *Saperda tridentata*. They flourished on this, but since the supply was soon exhausted, substitute foods had to be used. Different substances were tried with varying success, but macerated beetles placed in water seemed to be the most satisfactory. Nematodes in cultures without food usually did not live longer than two days. The presence of food acted as a stimulant to copulation and oviposition, but both varied directly with the abundance and adaptability of the food.

The nematodes seemed to show no preference to either day or night for depositing their eggs or any other of their habits.

LENGTH OF ACTIVE BREEDING STATE.—If the nematode is considered to be mature from the time of mating, it spends an average of about two days as a normal active breeding adult.

## DIPLOGASTER AERIVORA

In 1856, Charles Lespés[1] gave a meager description of a nematode which he found parasitizing *Leucotermes lucifugus*. His description is short and so indefinite that it might apply to several species of nematodes, but the habits he discusses closely resemble those of the nematodes found in *L. lucifugus* in Kansas. However, Dr. Cobb identified this nematode as *Diplogaster aerivora* (fig. 2, *I–L;* 3) and described it as follows:

**Diplogaster aerivora,** n. sp. $\frac{.8\ \ 8.9\ \ 12.\ \ '51'^{62}\ \ 87.}{1.6\ \ 3.9\ \ 4.9\ \ 5.9\ \ 2.6}$ 1.5 mm. The transparent, moderately thin layers of the colorless naked cuticle are traversed by fine transverse striæ, resolvable with high powers under favorable conditions. The cuticle is traversed also by 24 longitudinal striæ. These longitudinal striæ are sometimes resolvable into quadrate elements, each consisting of four punctations arranged in a quadrangle whose width is equal to the width of the stria. In the majority of specimens these quadrate elements were not to be seen. The distance between the striæ varies in different parts of the body up to about twice their width. The striations of the cuticle, both transverse and longitudinal, vary within pretty wide limits, the varying

[1] Lespés, Charles. Sur un nématoïde parasite des Termites. *In* Ann. Sci. Nat. Zool., s. 4, t. 5, p. 335–336. 1856.

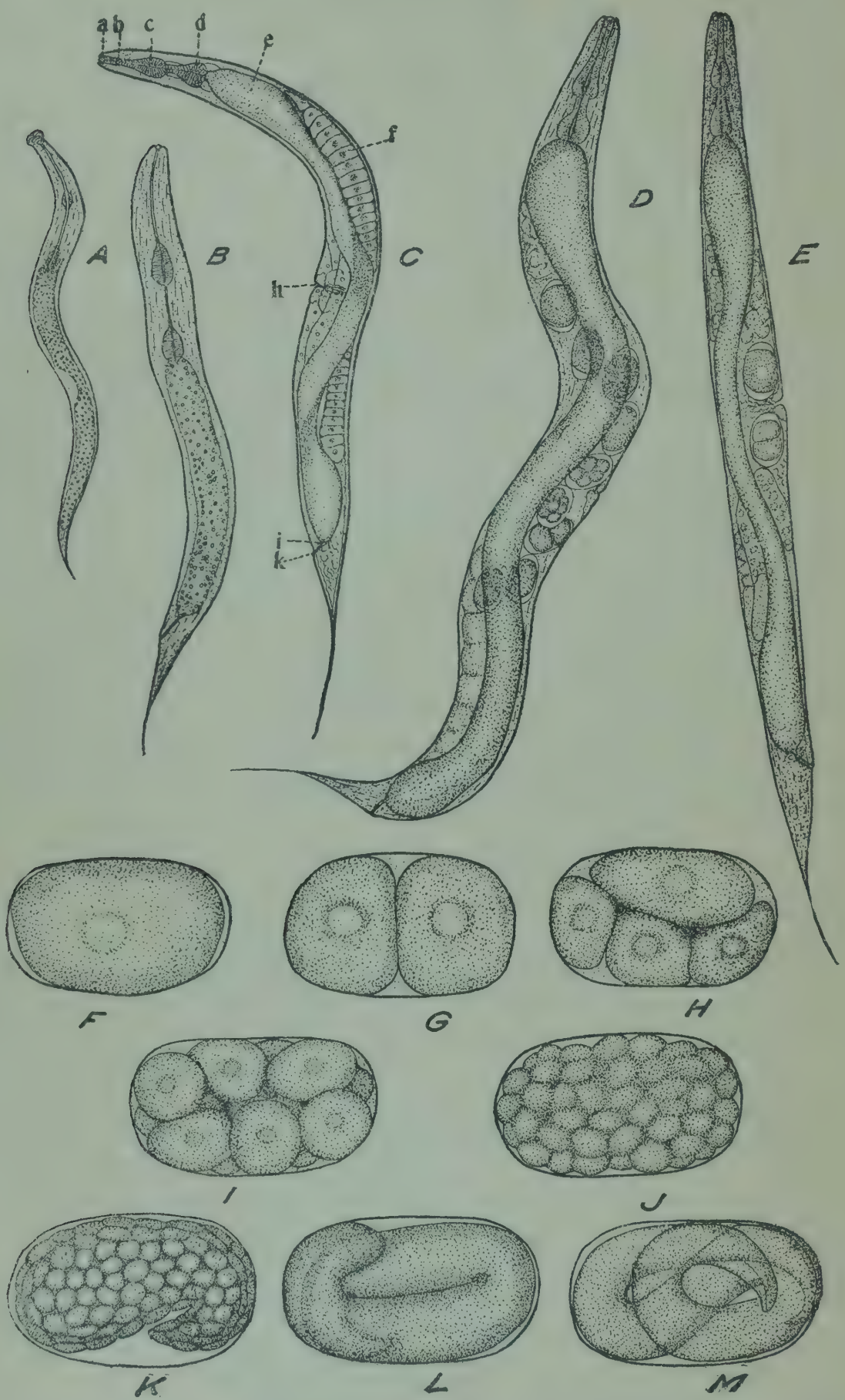


FIG. 3.—*Diplogaster aerivora: A*, Form found in termite (× 150); *B*, at time of hatching (× 400); *C*, female reared in water culture, not quite mature (× 100), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *f*, ovaries, *h*, genital pore, *i*, rectum, *k*, anus; *D*, mature female reared in moist soil (× 75); *E*, mature female reared in water culture (× 125); *F–M*, development of the egg (× 500). Drawings by A. L. Ford.

conditions evidently being a function among other things of the age or condition of the cuticle. There are lateral wings, though these consist simply of a pair of slightly modified longitudinal striæ.

The conoid neck becomes convex-conoid toward the truncated head, which is not set off in any way. There are six comparatively well amalgamated lips, each of which bears two innervated papillæ, one on the forward surface and somewhat forward pointing, and one on the outer surface and somewhat outward pointing. The anterior of these two papillæ is extended beyond the surface of the lip in the form of a minute seta or innervated papilla, and corresponds to the cephalic seta of other species of Diplogaster. The contour of the lip is not much disturbed by the presence of the posterior papilla, which is sometimes very difficult to see. Close behind the lateral papillæ or setæ there are minute openings in the cuticle, which in character closely simulate the amphids in some other species of Diplogaster, notably those of *D. fictor*. No doubt these are really the outward expression of minute amphids. Distally the lips have thin extensions which can close together over the pharynx in such a fashion that the front of the head is comparatively flat, though the tips of these lips may be recurved and point forward so as to make an exceedingly minute elevation at the middle of the front of the head. The latter has its front surface on the whole very slightly depressed.

The pharynx is about as deep as the front of the head is wide, and bears near its base on the dorsal side a relatively large, rather acute movable conoid tooth or onchus, which reaches about one-third the distance to the lips when the latter are closed, but which is relatively farther forward when the mouth is open. In addition there is a very much smaller submedian projection that undoubtedly may be denominated a rudimentary onchus. When the lips are closed the pharynx is a little wider at the base than anteriorly. At the base of the lips, opposite the posterior circlet of labial papillæ, the width of the pharynx is a little more than one-third that of the corresponding part of the head. Posteriorly, however, the width appears to be nearly three-fifths that of the corresponding portion of the head, at least when the head is viewed in profile. The walls of the pharynx are thin but refractive and fairly well ceratinized. The surface of the dorsal onchus is more highly ceratinized than that of other portions of the pharynx. Both the onchus and the wall of the pharynx have a yellowish or brownish color like that of the spicula. The end of the esophagus receives the base of the pharynx and is at once fully two-thirds as wide as the corresponding portion of the head. It continues to have the same diameter for some distance, then begins to expand and continues to do so to somewhat behind the middle of the neck, where it rather suddenly diminishes in diameter in such a way that it is proper to speak of a median bulb, although the anterior end of this bulb is not very distinctly set off by constriction from the anterior esophageal tube. This bulb contains an elongated valvular apparatus which is about one-third as wide as the bulb itself. This latter is three-fourths as wide as the corresponding portion of the neck. Notwithstanding the rather massive character of this median bulb, the succeeding portion of the esophagus is only about one-fourth as wide as the corresponding portion of the neck. However, it soon begins to widen and forms a somewhat pyriform cardiac bulb three-fourths as wide as the base of the neck. This bulb does not contain any very evident valvular apparatus, though in it there are faint indications of a modification of the esophageal lining. The intestine joins the posterior surface of the cardiac swelling, and at this point is about one-third as wide as the corresponding portion of the body. There is no very distinct cardia. The intestine widens out rather gradually and attains a width at least half as great as that of the body.

The tail end of the female begins to taper from some distance in front of the anus. This latter is slightly raised, especially its broader posterior lip. Behind the anus the tail diminishes somewhat more rapidly for a short distance and thereafter tapers regularly to the hairfine terminus. From the anus the rectum, which is about as long

as the anal body diameter, extends inward and forward. Nothing definite is known with regard to the lateral fields.

From the well-developed, slightly depressed vulva the vagina leads inward at right angles to the ventral surface halfway across the body, where it joins the two symmetrically placed uteri. The internal female organs are double and reflexed, and the ovaries, which are rather narrow and packed with small ova arranged irregularly, reach back to the vulva or even beyond. The ellipsoidal eggs are about as long as the body is wide and about two-thirds as wide as long. Their shells are smooth and rather thick. Specimens have been seen in which well-developed embryos existed in the eggs contained in the uteri. Other specimens have been found in which two to three dozen embryos had escaped from the eggs and then devoured the whole interior of the mother's body. The excretory pore is located opposite the cardiac swelling.

Male formula. $\frac{.9 \quad 11. \quad 15. \quad 'M^{68}. \quad 89.}{2.2 \quad 5.4 \quad 6.1 \quad 10. \quad 4.6}$ 0.8 mm. The tail of the male diminishes suddenly in diameter from the raised anus in such fashion that at a distance from the anus not very much greater than the anal body diameter it has a diameter only about one-fourth to one-fifth as great as at the anus. At this point, which is immediately behind the posterior group of male papillæ, the tail begins to taper rather gradually and somewhat uniformly, and continues so to do to the hairfine terminus, though there is at first a very slight increase in the diameter, so that the tail has the appearance of being very slightly constricted just behind the posterior caudal group of male papillæ. There is no spinneret, and there are no caudal glands. The two equal, rather slender, tapering, arcuate, brownish, acute spicula are about one and one-half times as long as the anal body diameter. At their widest part, a little distance behind the cephala, the spicula have a width about one-tenth as great as that of the corresponding portion of the body. From this widest part they taper gently toward the cephalated proximal ends. In the other direction the spicula taper regularly to their acute terminals. The accessory pieces surround the spicula at their distal extremities. The portion of the spiculum surrounded by the accessory piece constitutes about one-sixth of the length of the former. Extending backward from this encircling part of the accessory piece is a median arcuate portion arranged nearly parallel to the spicula and having its proximal end somewhat cephalated. The entire length of the accessory piece, including this median dorsal portion, is about one-third that of the spicula. Like the spicula the accessory pieces are brownish in color.

The hemispherical-conoid innervated supplementary male organs are located as follows: In front of the anus three pairs, two of which are ventrally submedian and one sublateral; the sublateral pair is nearly opposite the middle of the spicula, and is on nearly the same zone as the posterior of the two ventrally submedian pairs; the anterior submedian pair is a little in front of the proximal ends of the spicula. Behind the anus the papillæ are arranged as follows: One pair subventral or ventrally submedian immediately behind the anus, two pairs sublateral, and three closely approximated pairs of small size, subventral. This latter group of three pairs is slightly farther behind the anus than the foremost preanal pair is in front of it. The three pairs do not appear to be uniform in structure, the two anterior appearing to be mere innervations, while the posterior one is a distinctly raised innervated papilla like the preanal ones. The posterior of the two pairs of sublateral postanal papillæ is a trifle in front of the group of three just mentioned, while the anterior is about halfway between the group of three and the anus. The anterior border of the anus constitutes a sort of rudimentary flap with an innervation. The testis is single and rather broad and tubular. It extends forward and is reflexed a short distance behind the base of the neck. The reflexed narrower part of the testis is about twice as long as the corresponding body diameter.

Habitat: Manhattan, Kans. Found feeding on grasshopper eggs after the eggs had been deposited in the ground.

The eggs of *Diplogaster aerivora*, which are elliptical in shape, averaged about 0.062 mm. in length and 0.0335 mm. in diameter. When freshly deposited, they were dark brown in color, but became transparent as the embryo developed. Segmentation often began before the eggs were deposited and the succeeding cell divisions could (fig. 3, *F–M*) be readily followed throughout. The eggs were numerous and could be found lying close together in groups of from about 6 to 30. The eggs hatched in about 18 hours from the time segmentation was first noticed. Toward the end of the egg stage the living worm (fig. 3, *M*) could be plainly seen moving about within the egg wall. These movements became more active until the worm finally ruptured the wall and escaped.

At the time of hatching, the young nematodes (fig. 3, *B*) of this species averaged 0.2145 mm. in length. At this stage the sex organs could not be distinguished, because of their poor development. In water cultures the worms grew very rapidly and reached maturity in three to four days. The females matured slightly in advance of the males (fig. 2, *J*). *D. aerivora* never exceeded 0.5 mm. in length nor completed its life cycle while within the termite (fig. 3, *A*). The nematodes remained in the termite in this form for an indefinite length of time, but upon emerging into moist soil they matured in about two days.

Although molting occurred in this species as in *D. labiata*, it was much more difficult to observe; and, while it was not observed more than once in any individual, it is probable that more molts did occur. Molting required less time in *D. aerivora* than in *D. labiata*, and the posterior end of the nematode remained free throughout the process.

In the older water cultures the adults became so numerous that they appeared as a living mass to the naked eye. The females, which were much larger than the males, averaged 0.99 mm. in length and 0.067 mm. in diameter, while the males averaged 0.75 mm. in length and 0.046 mm. in diameter. When free in moist soil, the worms became even larger; the females (fig. 3, *D*) averaged 1.632 mm. in length and 0.1192 mm. in diameter, and the males (fig. 3, *E*) averaged 1.1425 mm. in length and 0.0724 mm. in diameter.

When reared in water cultures, the females appeared darker than the males, but when found in the soil both sexes appeared pearly white. The alimentary canal of the female, like that of *D. labiata*, was spiral, while that of the male was straight. The posterior end of the female's body tapered into a long, threadlike process, but in the male this process was shorter and its body ended in an abrupt hook.

Process of mating.—The process of mating in *D. aerivora* (fig. 2, *L*) was much the same as in *D. labiata*. The male clasped the female slightly back of the middle of the body, so that its anal opening was in direct apposition to the genital pore of the female. In mating, the posterior end of the male usually completely circled the body of the female, although exceptions occurred. Although many instances of mating

were observed, none lasted over 4½ minutes. As the mating neared completion, the female became more active and broke free.

Relation and economy of the sexes.—Both males and females mated repeatedly with different individuals. A single female was observed to mate with 7 different males, and during this time laid a total of 317 fertile and 14 infertile eggs. The length of time from the first to the last mating was 13 days. The greatest number of fertile eggs produced from a single mating by any individual under observation was 125, but the average number was 52.63. A single male was successfully mated with 10 different females, the latter depositing 624 fertile eggs. The total time which elapsed during these 10 matings was 19 days.

Time and method of oviposition.—A single instance was observed of a female depositing a fertile egg 30 minutes after mating, although from one to two hours are usually required. The eggs developed in the ovaries in large numbers and were rapidly discharged through the genital pore. With age the females became very sluggish and did not appear to be able to discharge their eggs; consequently these eggs hatched within the body of their parent, where they fed on her internal organs. Usually they were unable to escape, although instances were observed where they escaped through the genital pore of the mother (fig. 2, *K*).

Proportion of sexes.—Three hundred specimens were examined, and of these 138 were males and 162 were females. In all cultures the females seemed to be more abundant.

Habits.—These nematodes were found parasitic in the heads of *Leucotermes lucifugus*, where under natural conditions the number varied from 0 to about 75. Where heavy infestation occurred, the termites became sluggish and often died. These worms were usually more numerous in the immediate region of the mouth parts of *Leucotermes lucifugus*, although it was not uncommon to find them in the upper part of the cavity of the head. A great many termites were dissected, and in no case were nematodes found in the abdomen. In infested colonies nematodes were often seen in the surrounding soil. These usually were found in masses, feeding upon the bodies of dead termites or other available decaying matter. Specimens of *D. aerivora* placed in water cultures were found to flourish in the same food that was used for *D. labiata*. It was necessary to feed these nematodes each day, for without food they died in a very short time. As in *D. labiata*, the presence of food appeared to stimulate copulation and consequently caused an increase in oviposition.

So far as could be determined, these nematodes showed no preference to either day or night in mating, oviposition, or other habits.

Length of active breeding stage.—The active breeding life of the female extended over a period of about 13 days, while that of the male was about 19 days. The complete life cycle of *D. aerivora* required from four to five days. As the individuals of this species which were

examined had no hibernation stage, their life cycle was continually repeated under favorable conditions. Insufficient moisture and lack of suitable food seriously interfered with the development of these nematodes.

A series of experiments was carried on to ascertain whether it is possible to introduce these parasites into *Leucotermes lucifugus*. Good cultures of nematodes were obtained in moist soil, into which specimens of *L. lucifugus* were placed. After two days a number of these termites were dissected, and it was found that there was an average of 22.9 nematodes in each head. In three days this average rose to 32.9 and in four days it was 46.6. In each instance the check count remained the same, being about 3 nematodes per head. After remaining in a similar culture for 12 days, all the termites died and the bodies were found to be literally alive with nematodes.

## SUMMARY

(1) The eggs of *Diplogaster labiata* hatched in from 30 to 32 hours, while those of *D. aerivora* hatched in about 18 hours.

(2) The eggs of *D. labiata* were deposited singly, while those of *D. aerivora* were deposited in groups.

(3) More cases of eggs hatching in the body were found in *D. aerivora* than in *D. labiata*.

(4) The eggs of both species developed similarly.

(5) Both species, when reared in water cultures, used the same food, but in nature they had different hosts.

(6) Both species molted, but the process differed in that *D. labiata* fastened its posterior end, while *D. aerivora* did not.

(7) The adults of *D. aerivora* were larger than those of *D. labiata* and required much less time to mature.

(8) In water cultures, the females of both species were more numerous than the males.

(9) Although mating was similar in both species, *D. labiata* required more time for the process.

(10) Individuals of *D. labiata* usually mated but once, while those of *D. aerivora* mated repeatedly.

(11) Neither species in their habits showed any preference to day or night.

(12) The females of *D. aerivora* had a period of oviposition of about 13 days, while in *D. labiata* this period lasted only about 2 days.

(13) In both species adaptable and plentiful food acted as a stimulant to reproduction.

(14) Both species attacked insects, but in different regions of the body, as *D. aerivora* was found in the head while *D. labiata* was found in the intestines.

(15) The life cycle of *D. labiata* required more than twice as much time as did that of *D. aerivora*.

(16) *D. aerivora* was successfully introduced into the termites.

# INSECT INJURY TO COTTON SEEDLINGS[1]

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## INTRODUCTION

The present work deals with leaf mutilation of cotton seedlings (*Gossypium* spp.) caused by insects. The observations were made in the vicinity of Tallulah, La., during the spring of 1915. Such injury to cotton seedlings is probably found throughout the entire area of cotton cultivation in the United States. The senior author has noted it in many parts of Texas, both the drier and more humid portions, in Louisiana, and in Arizona on irrigated cotton. Since these localities approximate the extremes of rainfall, temperature, and sunshine under which cotton is cultivated, it is reasonable to expect the injury at almost any place.

## CHARACTER OF INJURY

The injury varies much in appearance and intensity, but all of the examples which have come to the attention of the authors have certain more or less constant characteristics. This is frequently noticed as soon as the seedlings appear above the ground, although it may not appear until later. The time of the cessation is also variable, but it does not seem to continue after the plants reach a height of 10 to 12 inches and usually stops much earlier. In the vicinity of Tallulah this injury is seen from the first sprouting of the plants until the latter part of May.

The first appearance is characterized by irregular holes appearing in the cotyledons. These vary from small holes through the leaf or small marginal incisions to almost complete loss of the leaf. Following this the later leaves are attacked in the same manner, with all possible variations in the type and degree of the injury. In some cases the terminal bud may be lost.

## LABORATORY STUDIES

Efforts were made to secure growing plants at the earliest possible date. For this purpose cotton seed was planted in boxes and pots in the laboratory during the very early spring, but lighting facilities were so poor at this season that the plants failed to thrive. The first healthy seedlings which were secured sprouted in the laboratory hotbed March 16 from seed planted in the middle of February. Seed planted in another part of this hotbed on March 5 sprouted well a little later. This hotbed

[1] The investigations upon which this paper is based were conducted under the direction of Mr. W. D. Hunter, in Charge of Southern Field Crop Insect Investigations, Bureau of Entomology.

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was covered with glass during the night and was only opened during the warmer part of the day. The plants appeared perfectly healthy at all times and grew well.

Other plantings were made in the laboratory yard at intervals during March for studies under outside conditions. Later, seeds were germinated between layers of moist absorbent cotton and placed in pots containing soil sterilized by baking. These pots were then placed in large screen cages and the plants were allowed to grow under this protection.

The first injury was noted in the hotbed on March 31. These seedlings had sprouted March 16 and at this time were about 3 inches tall. They had been protected from cold by the glass covers, and the soil had been well manured. On this first morning a number of plants were found to have been injured.

Following this the progress of the injury was noted carefully. All plants were examined daily and those showing injury were tagged. In this manner a record of the number of plants injured each day was secured. On the morning of April 14 nine new seedlings were injured; on April 15 five, on April 16 six, on April 17 two, and on April 18 three.

In order to determine the period in which the injury was incurred, both morning and evening counts were started. These showed the number of seedlings injured during the night and during the day. These observations were started April 22 and continued until May 4. The results are presented in Table I.

TABLE I.—*Comparison of day and night injury to cotton seedlings*

| Date of examination. | Number of seedlings injured during day. | Number of seedlings injured during night. | Date of examination. | Number of seedlings injured during day. | Number of seedlings injured during night. |
|---|---|---|---|---|---|
| Apr. 22 | ..... | 5 | May 1 | 0 | 1 |
| 23 | 7 | 6 | 2 | 0 | 3 |
| 26 | 4 | 10 | 3 | 0 | 4 |
| 27 | 2 | 2 | 4 | 0 | 3 |
| 28 | 4 | 5 | | | |
| 29 | 4 | 4 | Total | 26 | 49 |
| 30 | 5 | 6 | | | |

From this table it is seen that 66 per cent of the injury appeared during the night and 34 per cent during the day.

On April 14 this same type of injury appeared upon seedlings which had just sprouted in the laboratory garden, and from that time it appeared about as abundantly here as in the hotbed.

The rapidity with which the injury was produced was quite striking, and special studies were made upon this point. A number of apparently healthy and entire seedlings were examined morning and evening, and in that way the amount of injury produced in a single night was

determined. This was done in both the hotbed and the garden, and the results were the same in both cases. Leaves which were entire and uninjured at nightfall would show large holes often occupying one-half of their area on the following morning. Later observations have shown practically entire leaves disappearing in the same manner during the night.

During the first few days when the injury was appearing in the hotbed a number of examinations were made during the daytime in the attempt to find some insect producing the injury, but not a single individual which could be suspected of being the cause was noted. However, on April 6, 50 square inches of the hotbed soil were examined to a depth of 3 inches, and 12 cutworms were found. If this was a fair sample of the hotbed, the soil there certainly contained hundreds of the worms. Eleven of these larvæ were very small, while one was about an inch in length.

The presence of these larvæ in the hotbed and the fact that they were known to feed upon plant leaves made it seem quite possible that they were responsible for more or less of the injury. Consequently several examinations were made at night, and a number of cutworms were found feeding on the leaves of the plants. At this time the same injury was noted on clover and weed leaves in the hotbed.

Several half-grown cutworm larvæ collected on cotton in the garden and hotbed were placed on the surface of the soil in a pot containing a number of seedlings. This pot was placed in a screen cage and the larvæ attacked the seedlings at once. Plate XII, and Plate XIII, figure 1, show several seedlings injured by these larvæ.

## STUDIES OF CLIMATIC FACTORS

A number of tests were conducted to determine whether any of the injury could be due to the exposure to low temperatures during the night or to the hot sunlight in the morning before the plants had time to become warm. In the first test a wooden frame was erected over a cotton planting in the laboratory garden just prior to the sprouting of the plants. This frame was 2½ feet in height and was covered with 8-ounce duck. This cloth was placed over the frame at sundown each day and allowed to remain until about 10 o'clock the following morning. In this manner the radiation was reduced under this cover during the night and the plants were protected from sudden exposure to the sunlight in the early morning. A minimum thermometer was suspended under the cover in the center of the bed about 15 inches from the ground and another was suspended at the same height in the open garden a few feet away. Records continued for a few nights showed only a slightly higher temperature under the shelter, so the frame was lowered to within 1½ feet of the ground and the thermometers were lowered to 6 inches. Following this the minimum temperatures under the cover usually ranged a few degrees higher than in the open.

This frame was first erected April 26 and on April 30 the first seedlings appeared above the ground. Of the 18 which sprouted this first day, 5 showed injury. On May 1, 9 of the 45 seedlings showing above the ground were injured, while on May 3, 10 out of 50 were injured. On May 4, 16 out of 70 and on May 5, 22 out of 70 were injured. These observations were continued until May 8 and new seedlings were injured practically every day.

On May 8 a second test of the same sort was started. In this case, however, the cotton row was covered just before sprouting with heavy pasteboard boxes, 1 foot square and 8 feet long. These boxes were covered with several layers of 8-ounce duck and were only removed from over the plants during the hotter part of the day. Minimum thermometers were arranged under the boxes and in the open in the same manner as that just described in the preceding test. In this case considerable differences in the nightly minimum temperatures were noted. It was usually from 3 to 6 degrees warmer under the box than in the open. On May 12 the first seedlings appeared, and of the 39 in sight, 3 showed injury to the leaves. This test was continued six days longer and the injury continued to appear.

For comparison with the seedlings growing in the garden and hotbed, a number of seeds were planted at intervals in pots and crocks containing soil sterilized by baking. Part of these were allowed to remain exposed in the open, while others were placed in screen cages. In the hundred or more seedlings grown in this manner not a single sign of injury was found, whereas the injury was appearing abundantly on plants growing in the garden and hotbed at this same time. From this it seemed quite evident that the cause of the injury was located in the soil which had not been baked.

## FIELD OBSERVATIONS

As the injury was appearing in the various fields at this same time, efforts were made to learn its extent and to discover any insects which might cause the lesions. In these studies all insects which were known to be leaf feeders were noted and an attempt was made to secure positive samples of their injury to cotton. On April 19 four small lepidopterous larvæ were found feeding upon the leaves of cotton seedlings at a plantation near Tallulah. The injury which they were producing was apparently identical with that already noted. These larvæ belong to the family Liparidae and are commonly known as "tussock moths" (*Hemerocampa leucostigma* Smith and Abbot). On this same date three larvæ of the same species were found feeding on the seedlings in the hotbed and one was found in the laboratory garden. Following this the field examinations showed a considerable number of these larvæ to be present around Tallulah, and associated with them were found several species of cutworms and "measuring worms." All produced nearly the same type of injury to the seedlings.

In order to determine definitely the amount of injury present in the various cotton fields around Tallulah and also the prevalence of the worms, a considerable number of examinations were made during the latter part of April. In these observations only the worms found on the cotton seedlings were noted. In order to make the figures more accurately represent the condition of the field, the plants were examined in groups of 100 each in all parts of the field. The results are summarized in Table II.

TABLE II.—*Records of examinations for insect injury to cotton seedlings in fields around Tallulah, La.*

| Date. | Number of seedlings examined. | Number of seedlings injured. | Per cent of seedlings injured. | Number of lepidopterous larvæ found. | Type of soil. | Remarks. |
|---|---|---|---|---|---|---|
| Apr. 20 | 1,000 | 266 | 26.6 | 25 | Sandy | All tussock larvæ; very small. |
| 21 | 200 | 30 | 15.0 | | ...do | Seedlings just above the ground. |
| 22 and 23 | 2,300 | 534 | 32.2 | 25 | ...do | Eleven cutworms and 14 tussock larvæ. |
| 22 | 1,000 | 84 | 8.4 | 1 | Buckshot | Cutworm. |
| 22 | 800 | 188 | 23.5 | 1 | Sandy | Do. |
| 22 | 800 | 54 | 6.7 | | Buckshot | |
| 24 | 2,300 | 207 | 13.4 | 9 | Sandy | Six cutworms and 3 tussock larvæ. |
| 27 | 1,200 | 380 | 31.7 | 4 | ...do | Two geometrid larvæ and 2 tussock larvæ. |
| 27 | 1,000 | 227 | 22.7 | 1 | ...do | Tussock larva. |
| 29 | 400 | 43 | 10.7 | | ...do | |
| Total | 11,000 | 2,013 | | 66 | | Forty-five tussock larvæ, 19 cutworms, and 2 geometrids. |
| Weighted average | | | 18.3 | | | |

From this it is seen that the percentage of plants injured at the various plantations visited ranged from 6.7 to 32, with an average of 18.3 per cent for the 11,000 seedlings examined. In the course of these observations 66 lepidopterous larvæ in all were found. By far the greater part of these were the "tussock" larvæ and the remainder were either cutworms or "measuring worms."

The possibility of the soils having some influence upon the extent of damage was considered, but the writers were unable to secure sufficient information to allow definite conclusions. Soils in the vicinity of Tallulah may be roughly classed as either "sandy" or "buckshot." The former is the light, sandy land found on the bayou fronts, while the "buckshot" is the dark, heavy, stiff "back land." Under boll-weevil conditions "buckshot" land is not adapted to cotton culture; hence, only two fields of this type of soil were located for study. The percentage of injured seedlings in these two fields was 6.7 and 8.4. These were the lowest records made and are considerably below the average of sandy fields near by. Whether or not this lesser degree of injury was due to the soil is open to doubt. Owing to the "coldness" of "buckshot" land in the spring, the cottonseed germinates slowly and consequently the plants were considerably smaller

than those on sandy land. This may have caused the difference in the percentage of injury. However, only one suspected larva (a cutworm) was found in the two fields.

The different lepidopterous larvæ noted were all observed to be feeding upon the leaves. The tussock larvæ were much the more abundant and evidently produced a great deal of the injury. During the earlier examinations nearly all of these tussock larvæ were quite small. The injury produced varied somewhat with the size of the larva. The very small individuals fed only upon the epithelium of the lower side of the leaf and the injury was not visible from above. With a slight increase in size the larvæ started to feed through the leaf and at this stage produced the peculiar type of injury shown in Plate XIII, figure 2. Later the older larvæ (one-half to full grown) ate large holes in the leaves, and the injury could no longer be distinguished from that of the other species concerned. Plate XIII, figure 3, shows the injury produced by one nearly full-grown tussock larva when confined in a large screen cage with cotton seedlings growing in a pot.

About May 1 nearly all cotton fields under observation suddenly began to show greatly increased injury until within a few days many fields had practically every plant more or less mutilated. This proved to be due to an invasion of grasshopper nymphs. These speedily became very abundant and swarmed over the young cotton, feeding principally upon the leaves. This is shown in Plates XIV and XV. These cotton leaves were collected in the field when the young grasshoppers were feeding upon them.

A little later in May the 12-spotted cucumber beetle, or adult of the southern corn rootworm (*Diabrotica 12-punctata* Olivier), became abundant locally and added to the injury. The work of these beetles closely resembled that of the worms and grasshoppers, though the holes made were usually not very large. At this same time woolly-bear larvæ began to appear in the fields and produced the same injury.

Following this great increase in injury to the plants caused by the grasshoppers, counts were made to determine the percentage of injured seedlings in four average fields near Tallulah. The information secured from these examinations is shown in Table III.

TABLE III.—*Abundance of injured cotton seedlings after the grasshopper invasion*

| Date. | Number of seedlings examined. | Number of seedlings injured. | Percentage injured. |
|---|---|---|---|
| May 14 | 800 | 792 | 99.0 |
| 15 | 3,500 | 3,446 | 98.5 |
| 17 | 2,000 | 1,920 | 96.0 |
| 17 | 1,000 | 1,000 | 100.0 |
| Total | 7,300 | 7,158 | ..... |
| Weighted average | ..... | ..... | 98.0 |

Here it is seen that 98 per cent of the 7,300 seedlings examined had been injured by some of the various agencies operating prior to that time. High as they are, these figures are representative of average conditions in the fields near Tallulah.

### ACTIVE PERIOD OF LARVÆ

On April 14 continuous examinations of cotton seedlings were made from 8 a. m. until noon and from 1 to 5 p. m. on two plantations near Tallulah. The day's records of worm collections were divided into hourly periods and in this manner the active time of the various larvæ was noted. The results of these studies are shown in Table IV. From this it is seen that the tussock larvæ were much the more abundant throughout the day and there seemed to be no time at which they were especially abundant on the plants. The same seems to be true of the other larvæ.

TABLE IV.—*Records of field examinations for larvæ by hourly periods on two plantations near Tallulah, La.*

| Period. | Number and kinds of larvæ found. | |
|---|---|---|
| | First plantation. | Second plantation. |
| 8 a. m. to 9 a. m. | 2 tussock larvæ, 1 cutworm | 1 tussock larva. |
| 9 a. m. to 10 a. m. | 7 tussock larvæ | 4 cutworms, 2 yellow "woolly-bear" larvæ. |
| 10 a. m. to 11 a. m. | 9 tussock larvæ | 9 tussock larvæ, 2 cutworms, 3 yellow "woolly-bear" larvæ. |
| 11 a. m. to 12 noon | 3 tussock larvæ, 2 small cutworms | No examinations. |
| 1 p. m. to 2 p. m. | 7 tussock larvæ, 1 small cutworm, 1 yellow "woolly-bear" larva. | No worms. |
| 2 p. m. to 3 p. m. | 6 tussock larvæ | 2 unknown larvæ. |
| 3 p. m. to 4 p. m. | 6 tussock larvæ, 1 small cutworm | 3 unknown larvæ. |
| 4 p. m. to 5 p. m. | 7 tussock larvæ, 2 yellow "woolly-bear" larvæ. | 1 tussock larva, 1 geometrid, 6 unknown larvæ. |
| Summary | 57 tussock larvæ, 5 cutworms, and 3 yellow "woolly-bear" larvæ. | 14 tussock larvæ, 8 cutworms, 5 yellow "woolly bears," 1 geometrid, and 11 unknown larvæ. |
| Total, both plantations. | 71 tussock larvæ, 13 cutworms, 8 yellow "woolly-bear" larvæ, 1 geometrid, and 11 unknown larvæ. | |

### INJURY TO TERMINAL BUDS

The greater part of the feeding of the insects just mentioned is confined to the leaves. However, a considerable number of plants were found with the terminal buds either partially or completely destroyed. Plate XVI, figure 1, shows the usual location of this injury. This seedling was found in the field with a lepidopterous larva embedded at the base of the bud (*a*). The small cavity where the larva was feeding is shown in the photograph. From this the injury progresses until often all the buds and small leaves above point *a* are eaten out.

## ULTIMATE EFFECT OF INJURY UPON THE PLANTS

The preceding pages have shown the different insects contributing to the mutilation of cotton seedlings, but it is the ultimate effect upon the cotton production of the plants which determines the economic importance of the injury. This is a point upon which it is difficult to secure accurate data, but a certain amount of information has been gathered by the writers.

A number of plants are evidently killed outright by the feeding of the insects; but this number appears to be so small, even in fields very heavily infested, that it is of no practical importance.

The leaf feeding is also of very doubtful importance. In severe cases it retards the growth of the plants somewhat and occasionally dwarfs them permanently, but usually they recover very rapidly, and there is no visible effect other than the slight retardation.

Apparently it is the injury to the terminal buds which produces the most important economic effect. When this bud is injured or destroyed, the development of the plant is greatly changed. Instead of having a single main stem extending to the top of the plant, two or more large branches develop just below the injured bud and serve as stalks to produce the fruiting branches. Usually several very abnormal clusters of leaves form around the stalk near the injury. In Plate XVI, figure 2, the result of similar injury is shown in comparison with a normal plant. These two plants were collected in the garden at the laboratory and were stripped of their leaves before being photographed. Plant *B* shows a normally developed stalk and its branches, while plant *A* shows the deformity caused by the destruction of the terminal bud.

About the middle of June a number of examinations were made in the fields near Tallulah in order to determine the abundance of these deformed plants. The results of these examinations are given in Table V.

TABLE V.—*Records of field examinations for deformed cotton plants at Tallulah, La.*

| Date. | Number of plants examined. | Number of plants deformed. | Percentage deformed. | Location. |
|---|---|---|---|---|
| June 8 | 4,000 | 314 | 7.8 | Plantation. |
| 9 | 100 | 3 | 3.0 | Hotbed.[1] |
| 9 | 100 | 7 | 7.0 | Laboratory garden.[1] |
| 10 | 1,000 | 87 | 8.7 | Plantation. |
| 11 | 100 | 4 | 4.0 | Do. |
| 11 | 500 | 63 | 12.6 | Do. |
| 16 | 400 | 33 | 8.2 | Do. |
| 17 | 600 | 42 | 7.0 | Do. |
| Total | 6,800 | 553 | ..... | |
| Weighted average | ..... | ..... | 8.1 | |

[1] Just prior to this examination the plants in the garden and hotbed had been hand thinned; and as the poorest plants were removed, the percentage of deformed plants was evidently greatly lowered.

From this it is seen that the percentage of deformed plants ranged from 3 to 10.6, with an average of 8.1. As these same fields furnished the records given in Tables II and III, and were shown in the latter to have practically every plant more or less mutilated, it seems evident that only a comparatively small amount of the injury produces final deformity. However, an injury which deforms only 8 per cent of the plants in a field still is of considerable importance.

When this deformity was first observed it was at once noted that the injured plants were not forming as many squares as normal plants of the same age and height. Further studies showed this effect to be so pronounced that counts were made in the fields to determine the relative squaring of deformed and normal plants. In these observations, every time a deformed plant was found its squares were counted, and likewise those on the nearest eight normal plants of the same size. The average of these normal plants was compared with the number upon the deformed one. In 40 cases out of the 229 recorded the squares on the injured plants exceeded the average of the nearby normal plants, but in all others the average of the normal ones was considerably higher than the number on the injured plants. A summary of these observations is given in Table VI.

TABLE VI.—*Effect of deformity upon fruiting of cotton plants*

| Date. | Deformed plants. | | | | Normal plants. | | | |
|---|---|---|---|---|---|---|---|---|
| | Number observed. | Total squares. | Average squares per plant. | Maximum squares per plant. | Number observed. | Total squares. | Average squares per plant. | Maximum squares per plant. |
| June 10..... | 87 | 248 | 2.8 | 10 | 700 | 3,804 | 5.4 | 16 |
| 11..... | 4 | 23 | 5.0 | 9 | 32 | 267 | 8.3 | 13 |
| 11..... | 63 | 52 | 0.8 | 6 | 502 | 1,105 | 2.2 | 10 |
| 16..... | 33 | 405 | 12.3 | 26 | 264 | 3,931 | 14.9 | 34 |
| 17..... | 42 | 559 | 13.1 | 34 | 336 | 6,122 | 18.2 | 53 |
| Total..... | 229 | 1,287 | ..... | ..... | 1,834 | 15,229 | ..... | ..... |
| Weighted averages | ..... | ..... | 5.6 | ..... | ..... | ..... | 8.2 | ..... |

The 229 deformed plants averaged 5.6 squares per plant, while the 1,834 normal ones averaged 8.2 squares. This gives a difference of 2.6 squares per plant in favor of the normal plants at the time of these observations.

From these figures it is evident that the necessity for the additional vegetative development before squaring retards the fruiting of the plants considerably. This is a point of great importance in cotton culture under boll-weevil conditions. The primary requisite for a successful crop in the presence of the boll weevil is early, rapid, and prolific fruiting. This allows the safe "setting" of a crop before the weevils multiply

sufficiently to infest all the squares. Hence, any agency which retards the formation of the squares in the early spring does a very serious injury to the crop. While the deformed plants may overtake the normal plants later in the quantity of fruit, this fruit will be produced too late to insure safe maturing.

Another effect of the deformity which may be of considerable importance is the ease with which the plants are split when the two or more branches fork at the same point. This gives a very weak stalk, and a comparatively slight jar will split it. In fact, the weight of a crop of bolls will break many of the plants.

## SUMMARY AND CONCLUSIONS

From the various observations discussed in this paper it seems that mutilation of cotton seedlings may be produced by any of several insect pests. These consist of a number of species of lepidopterous larvæ (cutworms, measuring worms, "woolly-bear" larvæ, tussock-moth larvæ, etc.), grasshoppers, and leaf beetles. In all fields several species of these pests were present, and in many fields all of them were found. During the spring of 1915 at Tallulah, La., the tussock larvæ were responsible for most of the damage early in the season and then were supplanted by the grasshopper nymphs. However, the relative importance of the various species undoubtedly varies with the locality and season.

Tests made with plants protected from low temperatures during the night and from bright sunshine in the early morning demonstrated that the injury would appear about as abundantly on these plants as on the unsheltered plants in the garden and field. Seedlings in large number, raised through this period in pots and crocks containing baked soil, failed to show the slightest trace of injury, although they were fully exposed to the weather.

Injury to cotton by cutworms has been known for many years, but usually has been considered to consist only of the cutting of the plant stem near the ground. In 1897 Howard [1] published a brief review of the information then available concerning these larvæ, but did not mention them as leaf feeders. In 1905 Sanderson [2] mentioned the injury due to these worms and also discussed the work of *Prodenia ornithogalli*. This species he recorded as being diurnal in habits and feeding upon the leaves, but he considered the damage to the squares and bolls as its most important injury. Sanderson also mentioned the "woolly-bears" as occasionally damaging cotton by feeding upon the leaves.

In actual effect upon the plants it seems that the injury of the various species may result in death of the plant, dwarfing of growth, or deformity

[1] Howard, L. O. Insects affecting the cotton plant. U. S. Dept. Agr. Farmers' Bul. 47, 32 p., 18 fig. 1897.

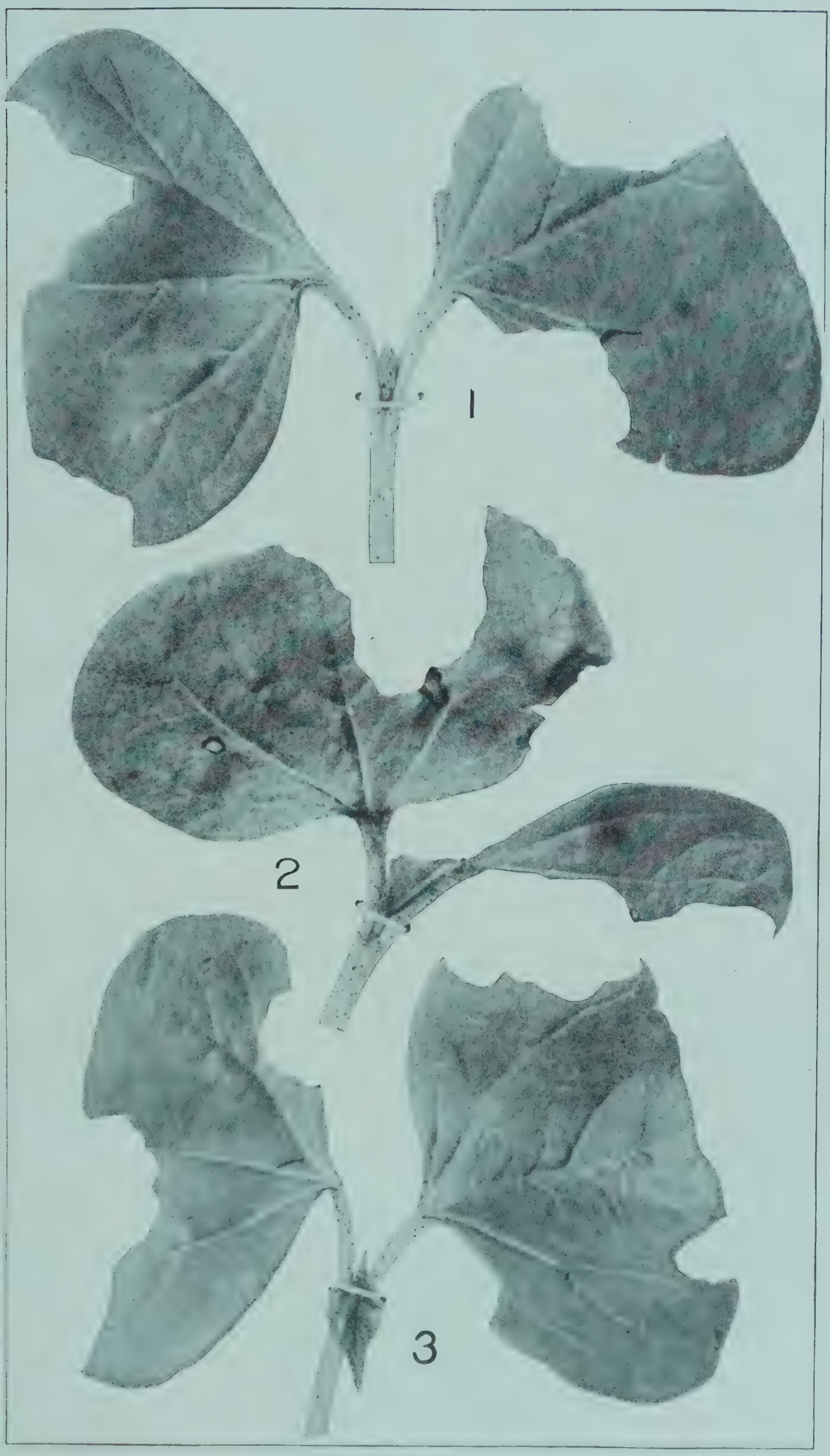
[2] Sanderson, E. D. Miscellaneous cotton insects in Texas. U. S. Dept. Agr. Farmers' Bul. 223, 24 p., 29 fig. 1905.

of the stem, producing retardation of the fruiting. Of these the deforming of the stalk is evidently much the more important. Field examinations have shown that an average of 8 per cent of the plants in the fields under observation were deformed and that these abnormal plants averaged 2.6 squares per plant less than the normal ones about the middle of June. As the cotton in these fields averages about 4 feet between the rows and is spaced about 18 inches in the drill, this would mean a loss of over 1,500 squares per acre at the critical period in cotton production in the presence of boll weevils.

The "woolly-bear" larvæ mentioned in this paper were reared and proved to be *Estigmene acraea* Drury. Two of the cutworms have been identified by Mr. S. E. Crumb, of the Bureau of Entomology, as *Prodenia ornithogalli* Guenée and *Peridroma margaritosa* Haworth, var. *saucia* Hübner.

## PLATE XII

Fig. 1.—Cutworm injury to cotton seedlings; produced in breeding cages.
Fig. 2, 3.—Cutworm injury to cotton seedling.


1
2
3

1
2
3

## PLATE XIII

Fig. 1.—Cutworm injury to cotton seedling.

Fig. 2.—Tussock larva feeding upon cotton leaf. The ragged injury shown here is usually produced by the smaller larvæ.

Fig. 3.—Injury produced by a nearly full-grown tussock larva when confined in a screen cage containing potted cotton plants.

PLATE XIV

Cotton leaves showing grasshopper injury.

1
2

1
2

## PLATE XV

Fig. 1.—Underside of cotton leaf showing grasshopper injury. This shows a number of places where the very small nymphs ate only the epithelium and did not penetrate the leaf.

Fig. 2.—Cotton leaf showing grasshopper injury.

## PLATE XVI

Fig. 1.—Injury to terminal bud of cotton by lepidopterous larva. This worm was embedded at point *a*.

Fig. 2.—Two cotton plants from laboratory garden with leaves removed. Plant *A* shows the abnormal forking caused by injury to the terminal bud, while *B* is a normal stalk. The absence of fruit on plant *A* is due to the deformity.

a
1

2
A
B

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# A SEX-LIMITED COLOR IN AYRSHIRE CATTLE[1]

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## TYPES OF INHERITANCE AS RELATED TO SEX

Two general types of inheritance as related to sex exist, aside from the ordinary secondary sex characters. Sex-linked inheritance depends on the great mass of hereditary factors that have been shown to be linked in transmission to the sex-determining factors; while sex-limited factors follow the simple Mendelian scheme of inheritance, but show a reversal of dominance in the two sexes. Frequently these two latter terms are used synonymously, but since there is a distinction between the two classes of transmission, and since the term "sex linked" is so much more descriptive of the hereditary phenomena to which it has been applied than is the term "sex limited," the foregoing terminology is used.

## HISTORICAL REVIEW

The classical case of sex-limited inheritance was reported by Wood (7), who made reciprocal crosses of the Dorset sheep, a breed horned in both sexes, with the Suffolk, a breed polled in both sexes. All $F_1$ individuals were the same, so far as the type of cross was concerned, the males being horned and the females polled. In the $F_2$ generation the fact that dominance differed in the two sexes resulted in three males being horned to one being polled, and three females being polled to one being horned.

Similarly in 1912 the writer reported a pair of rudimentary teats in swine, located on the lower part of the scrotum of the male and on the inner thighs of the female, behind the inguinal pair, which presented the same phenomenon in transmission, the character being dominant in the male and recessive in the female.

Gerould (2)[2] reported in 1911 on the inheritance of yellow and white in the common clover butterfly (*Colias philodice*). White is dominant to

[1] Paper No. 3 from the Laboratory of Animal Technology, Kansas Agricultural Experiment Station.
[2] Reference is made by number to "Literature cited," p. 147.

yellow in the female, but it is recessive in the male. Something lethal seems to be connected with homozygosis for white; hence, white as a somatic character appears only in the female. The yellow female is YY, the white female YW. Males are either YY or YW, but are always yellow.

Jacobson (3) made some observations on *Papilio mennon* L., which were studied from a Mendelian standpoint by De Meijere (5) in 1910. There are three varieties of females in this species known as Achates, Agenor, and Laomedon, respectively, in the order of their dominance. The males corresponding to these three forms are all alike, although each of the female patterns may be carried in a recessive manner. Furthermore, De Meijere believes that the female carries the male pattern homozygously; but, owing to the reversal of dominance, the male character never becomes somatic. The Laomedon probably represents the female expression of the male condition. The principal difference between this and the previous cases is that the changes in dominance affect the homozygotes as well as the heterozygotes.

## AYRSHIRE BLACK-AND-WHITE

A case which seems to fall under this general sex-limited group is found in the inheritance of black-and-white as alternative to red-and-white in Ayrshire cattle. While the general breed color is red-and-white, black-and-white animals have been known for some time, as shown by Kuhlman (4). Practically no attention has been paid to the mode of inheritance of this color, since in America it has been considered undesirable and selection against it has been practiced. It is difficult to state whether the black is due to a true black pigment or whether it is simply a very dense red. Under the microscope typically black granules seem to be present, but no chemical solutions of the pigments have yet been attempted.

## SOURCE OF THE DATA [1]

The Ayrshire herd bull at the Kansas Experiment Station, Melrose Good Gift, is a very deep mahogany-and-white; in fact, the black-and-white previously referred to. It is through the study of his ancestry and breeding performance, the ancestry and breeding performances of the cows in the herd, including the black-and-white animals, and the records of some of the former herd bulls that the present data were secured. In all, 63 individuals were included. Much larger numbers might have been obtained by adding the progeny of red-and-white males and females to the table; but since they demonstrated no facts different from those here included, their records are not presented.

[1] Acknowledgments are hereby made to Prof. O. E. Reed, of the Department of Dairy Husbandry, Kansas Experiment Station, for facilities extended in obtaining the data.

### PROGENY OF MELROSE GOOD GIFT FROM RED-AND-WHITE COWS

Fifteen red-and-white cows in the herd were mated to Melrose Good Gift to produce 20 calves, of which 10 were black-and-white bulls and 10 were red-and-white heifers. All of the bulls were as red as the heifers at birth, but at 2 to 4 months of age the blackish tinge began to develop, and within 4 months the youngsters became distinctly black-and-white. The heterozygous male progeny of Melrose Good Gift differed from the homozygous male progeny in that the black tinge developed more slowly and also became much less intense on maturity. While in the mature homozygous bull the black is very distinct throughout the pigmented areas, in the mature heterozygous bull the black may appear only as a streaked border where the pigmented spots adjoin the white, or at the limbs, muzzle, ears, and tail. The main portions of the colored parts of the animal are usually a very dark red which blends gradually, although in a particulate manner, into the blacker borders. The heterozygous heifers are red-and-white, and while occasional dark hairs are found, no regular means whereby the heterozygous red-and-white females could be distinguished from the homozygous red-and-white females was discovered. It should be further noted that the black color of the homozygous female is by no means as intense as that of the male, although the black is indisputably present.

### HETEROZYGOUS BLACK BULLS TO HOMOZYGOUS RED COWS

Johanna Croft King, College Marquis, Sir Croft of Spring City, Woolford's Good Gift, and Lessnessock Oyama's Good Gift were bulls which by their breeding performance and somatic description must have been heterozygous for the black factor. The last two bulls were found in the pedigree of Melrose Good Gift, while the first three were used at one time or another at the college as herd bulls. Records of these in matings to homozygous red-and-white cows were available for all except Woolford's Good Gift, and the result showed four red-and-white heifers, four black-and-white bulls, and 5 red-and-white bulls. This is the most probable distribution of colors in both the males and females and is perfectly in alignment with the interpretation of the method of inheritance as given.

The reciprocal cross of red-and-white bulls to black-and-white cows gave two black bulls to one red bull and two white heifers, also the most probable expectation.

### BLACK-AND-WHITE COWS MATED TO RED-AND-WHITE BULLS

Only three calves were available from this type of mating, all red-and-white daughters of Bangora, the original black-and-white cow in the herd. While the numbers are too small to be conclusive, yet they conform to the expectation.

## RESULTS OF THE DIFFERENT CROSSES

If the factor for the black-and-white color is represented by B, the hereditary constitutions are as follows: BB is always black-and-white; bb is always red-and-white; Bb is always black-and-white in the male and red-and-white in the female. All of the nine possible matings were discovered, as shown in Table I.

TABLE I.—*Results of nine possible matings of Ayrshire cattle*

| Sires. | Dams. | Male offspring. | | Female offspring. | |
|---|---|---|---|---|---|
| | | Black-and-white. | Red-and-white. | Black-and-white. | Red-and-white. |
| BB | BB | 1 | 0 | 3 | 0 |
| BB | Bb | 0 | 0 | 0 | 1 |
| BB | bb | 10 | 0 | 0 | 10 |
| Bb | BB | 3 | 0 | 2 | 1 |
| Bb | Bb | 1 | 0 | 1 | 0 |
| Bb | bb | 4 | 5 | 0 | 4 |
| bb | BB | 0 | 0 | 0 | 3 |
| bb | Bb | 2 | 1 | 0 | 2 |
| bb | bb | 0 | 7 | 0 | 9 |
| Total | | 21 | 13 | 6 | 30 |
| Expected | | 20.75 | 13.25 | 5.25 | 30.75 |

The expectations here presented are based on the most probable result of each of the matings, considered on an individual basis with reference to the number of animals produced by each type of mating, but without figuring the proportions of the sexes as equal. From these data it would appear that the black-and-white color of Ayrshire cattle behaves in an ordinary sex-limited manner similar to the horns in sheep as discussed by Wood (7) and the rudimentary mammæ in swine as reported by the writer (6).

## DISCUSSION

Arkell and Davenport (1) have reported on the inheritance of horns in sheep and have attempted to bring it under the ordinary sex-linked scheme of inheritance by an ingenious system of inhibitors and horn factors. Such an explanation was doubtless justified when horns in sheep were the only character known in which the reversal of dominance in the two sexes existed, but now that at least two other characters are known in which an exactly similar system of inheritance occurs, it seems unnecessary to assume the complexities hypothesized by these investigators. Instead, the much simpler and probably more perfectly descriptive explanation adopted by Wood (7) in his original paper seems more logical.

## COLOR RECORD OF PROGENY IN AYRSHIRE CATTLE

The following record presents the data considered in this paper. The term "red" refers to red-and-white and the term "black" refers to black-and-white. The hereditary constitution assigned the breeding animals retained in the herd or found in the pedigrees of animals in the herd is also given.

Johanna Croft King, Bb (described as dark).
- Sir Croft of Spring City, Bb (black).
- Johanna of Juneau, bb (red).

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College Marquis, Bb (described as dark).
- Marquis of Woodruff, bb (red).
- Maggie of Woodruff, Bb (red).

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Woolford's Good Gift, Bb (described as mahogany).
- Lessnessock Oyama's Good Gift, Bb (described as dark).
- Pearl 3d of Woolford, bb (red cow).

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Melrose Good Gift, BB (black-and-white).
- Woolford's Good, Gift Bb.
- Florence Melrose, Bb (red cow).

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College Maud, bb (red).....
- Marquis of Woodruff, bb (red).
- Star of Hillview, Bb (red).

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Bangora, BB (black).....
- White Prince, Bb (described as mahogany in pigmented areas).
- Star of Hillview, Bb (red).

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College Marquis 2d, bb
College Marquis 3d, bb } (red).....
- College Marquis, Bb (dark).
- College Maud, bb (red).

(See progeny of College Maud.)

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Progeny of College Maud 31350 (red), bb:
- One red heifer by unknown red bull, bb.
- One red heifer by College Marquis, Bb.
- Three red bulls by College Marquis, Bb.
- One red heifer by Johanna Croft King, Bb.
- One red heifer by Sir Croft of Spring City, Bb.
- One red heifer by Melrose Good Gift, BB.

Progeny of College Maud 2d (red), bb (daughter of College Maud by College Marquis):
- One red heifer by College Marquis, Bb.

Progeny of College Maud 2d's heifer (red), bb (daughter of College Maud 2d by College Marquis):
- One black bull by Sir Croft of Spring City, Bb.
- One red bull by College Marquis 3d, bb.
- One black bull by Melrose Good Gift, BB.
- One red heifer by Melrose Good Gift, BB.

Progeny of Kansas Croft Maud (red), Bb (daughter of College Maud by Sir Croft of Spring City):
- One red heifer by Melrose Good Gift, BB.
- One black bull by Cavalier's College Master, bb.

Progeny of Johanna Croft Maud (red), bb (daughter of College Maud by Johanna Croft King):
- One red heifer by Melrose Good Gift, BB.

Progeny of Georgie Em 25749 (red), bb:
One red heifer by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
One black bull by Melrose Good Gift, BB.
One red heifer by Melrose Good Gift, BB.
One red heifer by College Marquis 2d, bb.

Progeny of Georgie Croft (red), bb (daughter of Georgie Em by Sir Croft of Spring City):
Three black bulls by Melrose Good Gift, BB.

Progeny of Marquis Em (red), bb (daughter of Georgie Em by College Marquis 3d):
One red heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.

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Progeny of Johanna of Juneau 26290 (red), bb:
One black bull by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
Twins (one black bull and one red heifer) by Melrose Good Gift, BB.
One red heifer by College Marquis 2d, bb.

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Progeny of Elizabeth of Juneau 26292 (red), bb:
One red bull by Sir Croft of Spring City, Bb.
One red bull by College Marquis 3d, bb.
Two black bulls by Melrose Good Gift, BB.

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Progeny of Rose of Oakdale 26291 (red), bb:
Two red bulls by College Marquis 2d, bb.
One red bull by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Rosa Lee Melrose (red), bb (daughter of Rose of Oakdale by Melrose Good Gift, BB):
One red bull by Cavalier's College Master, bb.

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Progeny of Canary Belle 25748 (red), bb:
One red bull by Sir Croft of Spring City, Bb.
One red bull by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Melrose Canary Belle, (red), Bb (daughter of Canary Belle by Melrose Good Gift, BB):
One red heifer by Cavalier's College Master, bb.

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Progeny of Fearnot of Oakdale 26289 (red), bb:
One black bull by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One red bull by College Marquis 2d, bb.

Progeny of Lady Marquis Fearnot (red), bb (daughter of Fearnot of Oakdale by College Marquis 3d):
One red heifer by Melrose Good Gift, BB.

Progeny of Bangora 29700 (black), BB:
One red heifer by Marquis of Woodruff, bb.
One red heifer by College Marquis, Bb.
Two black heifers by College Marquis, Bb.
One black bull by Sir Croft of Spring City, Bb.
One black bull by Johanna Croft King, Bb.
One black heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Bangora 2d (black), BB (daughter of Bangora by College Marquis):
One black bull by Johanna Croft King, Bb.
Two black heifers by Melrose Good Gift, BB.

Progeny of Bangora's Melrose (black), BB (daughter of Bangora by Melrose Good Gift, BB):
One red heifer by Cavalier's College Master, bb.

## CONCLUSIONS

(1) Black-and-white color is a simple allelomorph of red-and-white color in Ayrshire cattle.

(2) In the male the black-and-white character is dominant and in the female the red-and-white character is dominant.

(3) Males heterozygous for the two characters are black-and-white, while females heterozygous for the two characters are red-and-white.

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# WATERMELON STEM-END ROT

[PRELIMINARY PAPER]

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During the last few years in certain parts of the United States shippers have been seriously troubled by a decay which attacks watermelons (*Citrullus vulgaris*) in transit and may sometimes destroy or render unsalable a large percentage of a shipment before it reaches its destination. Owing to this fact, in the season of 1915 the Department of Agriculture began a careful investigation of shipping conditions, in the course of which the present writer had an opportunity to make a laboratory study of some decayed material.

This material was taken from a shipment received in Washington, D. C., on July 24, 1915. The shipment consisted of five carloads of approximately 900 watermelons each, no one car of which yielded more than 300 salable melons, owing to the prevalence among them of the disease. The decayed watermelons were distributed through the car entirely without reference to position, a fact which made it seem manifestly impossible that the trouble could have originated from mechanical or chemical injury received from contact with the walls or the floor of the car.

This examination indicated, moreover, that, as has been reported in the case of other shipments, the injury of these watermelons had occurred in a very uniform manner. In its early stages the presence of the decay was indicated by a watery discoloration of the rind in an area closely surrounding and apparently extending from the stem. Beginning in this way there were all stages of decay up to those where about half or three-quarters of the melon were involved. In such cases the rind of this portion had become soft and wrinkled, so that in cross section it appeared much like that of the watermelons shown in the lower row of Plate XVII, figure 1. The meat below this part of the rind was slimy and blackened, while that at the opposite end of the melon remained sound, not having as yet become included in the decay. Owing to the warm, moist conditions at this season, the portion involved was covered by a gray or somewhat black mold, so that the origin of the trouble could not be readily ascertained.

An abundance of material being available at this time, an attempt was made to find out whether the injury was due to the action of some fungus, and, if this proved to be the case, to obtain the specific organism

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in pure culture. In endeavoring to obtain such cultures, the following procedure was adopted. Several watermelons were selected in which the decay was just beginning to be apparent. A razor was flamed; and with this, a funnel-shaped section, which included a portion of both diseased and healthy tissue, the two being separated by a more or less distinct line of demarcation, was cut from the melon. After the razor had been flamed again, the section was divided along the line of demarcation which distinguished the advancing edge of the decay, the plug being cut from the inside toward the outer surface. This gave access to a portion of the rind to which the fungus filaments were probably just advancing and which would be unlikely to contain concomitant forms. From this region, using a sterile platinum needle, small portions were removed from just below the surface and placed directly on synthetic agar in sterile Petri dishes. After two days, during which the plates were kept at a temperature of 27° C., an abundant mycelial growth of a gray color appeared in every instance. A number of transfers of the mycelium thus obtained were made to potato cylinders, and in all cases a fungus developed which possessed the characteristics of the genus Diplodia. In order to test the capacity of this organism for producing the decay, the pure culture was inoculated into a sound watermelon at three widely separated points, at each of which the characteristic rot was reproduced.

The direct connection between this fungus and the disease having been thus indicated, 16 healthy watermelons were obtained for more inoculations. They were bought at the wharf in Washington, D. C., and came from the Pyankatank River district in Virginia, a region free from the disease, so far as is known. It may be well to mention in this connection that the decay has usually been reported as occurring on the variety known as "Tom Watson." This is probably due to the fact that in the last few years this melon has been grown somewhat to the exclusion of other varieties. Of the melons chosen for inoculation, three were "Excel" melons; the remainder were of the "Tom Watson" variety.

These melons were placed on a table near a large window which was kept open the greater part of the day, and were protected from the direct light of the sun by a cardboard screen. For a period of nine days, during which time the melons were under observation, the average temperature was 26.5° C. Of these 16 watermelons, 8, two of which were of the "Excel" variety, were inoculated with the fungus, the cultures used in this case having been derived from the original subculture. This was accomplished by making with a sterile knife at a single point near the stem an incision, into which a bit of the growing fungus mycelium was introduced. A similar wound was made in the remaining 8 melons, including the third "Excel" variety, but no infectious matter was introduced. Within 36 hours the 8 inoculated melons began to show

signs of decay, while the 8 checks remained perfectly sound throughout the course of the experiment. There was no decay present on the inoculated melons except that which originated at the point of inoculation.

The decay is first noticeable as a somewhat circular discolored area surrounding and extending from the point of inoculation. On the watermelons observed in the laboratory this area gradually increased in size until at the end of six days about half of the melon was involved. At this time the advance of the decay seemed to become less rapid and the area which was first decayed began to show a blackening due to the formation of pycnidia by the fruiting fungus. This area spread daily, and at the close of nine days the stem end of the melon presented a withered, charred appearance. Plate XVII, figure 1, is a reproduction of a photograph of nine of these melons. The four in the upper row are checks; the five below were inoculated.

The fructification of the fungus may be briefly described as follows:

Pycnidia separate or confluent, smooth or, under moist conditions, covered with loose olivaceous hyphæ, 180 to 250$\mu$ in diameter. Spores 24 to 30$\mu$ by 10 to 14$\mu$, oval, uniseptate, dark brown. On the material taken from the watermelons inoculated in Washington no paraphyses could be detected. They are present, however, when the organism is grown upon potato cylinders, a fact which would tend to support the conclusions reached by Taubenhaus,[1] to whose work reference will be made in the following paragraph.

It has long been known that those members of the Sphaeropsideae which produce brown uniseptate spores are extremely variable. The distinctions between the genera Diplodia, Botryodiplodia, Chaetodiplodia, Lasiodiplodia, and Diplodiella have been based on slight structural variations in the pycnidia. The points of separation are the relation of the pycnidia to one another, whether scattered or cespitose; their relation to the host, whether subcutaneous, erumpent, or superficial; the presence or absence of bristles and of paraphyses. These are all characteristics which one might expect to vary somewhat with the characteristics or the condition of the host. This variation probably occurs; and for this reason there has been some uncertainty as to the proper position certain species should occupy in classification. *Botryodiplodia theobromae* Pat., which causes a dieback of *Hevea braziliensis* in Ceylon, southern India, and the Malay States, is an example; and in his account of this fungus Petch[2] remarks that—

> Among the names which are known to refer to this species are *Macrophoma vestita*, *Diplodia cacaoicola*, *Lasiodiplodia theobromae*, *Diplodia rapax*, and there are probably others. *Botryodiplodia theobromae* is its earliest name, as far as is known, but some prefer to call it *Lasiodiplodia theobromae*.

[1] Taubenhaus, J. J. The probable non-validity of the genera Botryodiplodia, Diplodiella, Chaetodiplodia, and Lasiodiplodia. *In* Amer. Jour. Bot., v. 2, no. 7, p. 324-331, pl. 12-14. 1915.

[2] Petch, Thomas. Physiology & Diseases of Hevea braziliensis . . . 268 p., 16 pl. London, 1911.

Taubenhaus, as a result of his inoculations upon sweet potato (*Ipomoea batatas*) with *Diplodia tubericola* E. and E., *Diplodia gossypii* Zim., *Diplodia natalensis* Pole Evans, and *Lasiodiplodia theobromae* (Pat.) Griff. and Maubl., suggests that the characteristics of the genus Diplodia be so extended that it may include all of the five genera.

This genus, although it is not thought to include forms which are absolute parasites, is nevertheless a source of serious trouble among some of our cultivated plants. The injury is usually confined to a fruit rot or to a dieback of the younger branches or shoots as in the Citrus disease prevalent in Florida and the Isle of Pines.[1] In both cases the fungus has been described as following an injury which has been previously inflicted either by mechanical means or as the result of the action of some other fungus. In the United States the more important crops which hitherto have been known to be affected are sweet potato, Citrus fruits, corn (*Zea mays*), and cotton (*Gossypium* spp.) In our Southern States the *Diplodia* injury is of considerable consequence in connection with these products. As one enters the Tropics the number of plants which are attacked increases. Among the list of hosts found here are *Citrus* spp., *Hevea* spp., *Theobroma cacao*, and *Thea* spp. In certain cases where the growing plant is attacked, the injury produced is sufficient to cause the death of the host, as is the case with *Diplodia vasinfecta* Petch, which causes an internal rootrot of tea.

Since the cotton, sweet-potato, and watermelon fields of the South are not widely separated, it is of some interest from the economic standpoint to know whether a species found on one host will grow equally well upon another. Plate XVII, figure 2, shows a watermelon nine days after it had been inoculated with a culture of *Diplodia tubericola* E. and E. obtained from Mr. L. L. Harter, of the Bureau of Plant Industry. The decay took the same course in this melon as has been described for the other inoculated material, which is shown in Plate XVII, figure 1. The pycnidia which were produced, however, retained the paraphyses.

While the *Diplodia* injury is apparently the cause of serious loss in the watermelon industry, there are other ways in which the crop suffers. Dr. W. A. Orton, Pathologist in Charge of Cotton and Truck Disease Investigations, Bureau of Plant Industry, who has made a careful study of shipping conditions, is inclined to believe that the injury is confined to certain districts. In other sections, anthracnose, due to *Colletotrichum lagenarium*, is the source of considerable trouble. To the losses thus caused by fungi must be added a small percentage of melons which have been damaged by rough treatment and by the use of cars which have been employed for the transportation of fertilizer or chemicals to the fields.

[1] Earle, F. S., and Rogers, J. M. Citrus pests and diseases at San Pedro in 1915. *In* San Pedro Citrus Path. Lab. 1st Ann. Rpt. 1915, p. 5–41, 19 fig. [1915.]

## PLATE XVII

Watermelons, showing the effect of inoculation with species of Diplodia:

Fig. 1.—The upper four melons were held as checks; the lower five are melons nine days after having been inoculated with a culture of *Diplodia* sp. which had been isolated from a decaying watermelon obtained from a freight car at Washington, D. C.

Fig. 2.—A watermelon nine days after having been inoculated with a culture of *Diplodia tubericola* E. and E.

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# EFFECT OF PASTEURIZATION ON MOLD SPORES

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## INTRODUCTION

Definite experiments to determine whether spores of the common saprophytic molds survive the temperatures used for the pasteurization of milk have not been reported. These spores are certainly present and are frequently abundant in ordinary market milk. Vague and general statements that such organisms do or do not survive are not uncommon, but are not supported by reference to actual work. To obtain such data studies were made with spores from pure cultures of a series of molds including several species of Penicillium, Aspergillus, and of the mucors, with, in some experiments, the addition of *Oidium* (*Oospora*) *lactis* and one strain of Fusarium. These sets of experiments were made to test, as carefully as laboratory conditions would permit, the temperatures used in pasteurization by the "holder" process, those used in the "flash" process, and the effects of dry heat.

## EXPERIMENTS WITH THE HOLDER PROCESS OF PASTEURIZATION

Bacteriological studies of milk treated by the holder process have fixed the temperatures between 140° and 145° F. (60° to 62.8° C.), maintained for 30 minutes, as the minimum heating for the destruction of pathogenic organisms which may be found in milk. Although certain bacteria survive this heating it has been found that milk so treated is free from the ordinary disease-producing organisms, safe for consumption, unchanged in taste, and low enough in acid organisms to be handled with-without souring too quickly.

To study the effect of this process of pasteurization on mold spores, conidia from pure cultures of molds were first transferred to tubes of sterile water to obtain a suspension of spores. Transfers from such a suspension reduce the danger of such spores being blown by air currents into the cotton plugs and upon the walls of the test tubes used, where they might escape the full temperature applied to the milk. In the first series the inoculations were made by transferring 1 c. c. of this suspension in sterile pipettes into duplicate tubes of sterile milk. In a later series a platinum loop was used, since the tendency of the conidia to float thickly upon the surface of the water made this a quick and effective method of handling them. For most species it was thus possible to transfer spores enough to make a visible film over a part of the surface of the milk. None

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of the species used produced visible growth except upon or near the surface of the milk. Observations of growth must include, therefore, the surface of the milk and especially the glass from the surface of the milk upward for a few millimeters, since most molds begin to grow first upon the glass. When no spores occurred upon the glass a free-floating colony in one case escaped observation until it fruited.

The inoculated milk tubes, with the exception of the control tubes, were heated in a water bath in which the water was agitated and the temperature of the milk was recorded in a control tube by a thermometer placed in the milk. The temperature in the tubes was not allowed to vary more than half a degree in either direction. The results of the experiments with the holder process are shown in Table I. In preparing this table the records of the checks, or unheated tubes, of successive experiments were found sufficiently uniform to permit them to be averaged and appear but once. Experimental tubes were made in duplicate; and when the results were not reasonably harmonious the work was repeated. Table I summarizes the tabulated data from a series of experiments extending over a period of several months.

TABLE I.—*Comparative effect of heating mold spores in milk to temperatures of from 120° to 150° F. (48.9° to 65.6° C.) for 30 minutes* [1]

| Name of mold. | Serial No. | Growth of spores when not heated (control). | | | Growth of spores when heated to temperature indicated and held for 30 minutes. | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 120° F. (48.9° C.). | | | 125° F. (51.7° C.). | | | 130° F. (54.5° C.). | | | 135° F. (57.2° C.). | | | 140° F. (60.0° C.). | | | 145° F. (62.8° C.). | | | 150° F. (65.6° C.). | | |
| | | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. |
| *Aspergillus candidus* | 106 | 0.2 | 0.5 | 0.7 | ..... | ..... | ..... | 0.1+ | 0.3 | 0.6 | 0.1+ | 0.3+ | 0.7 | (?) | 0.3+ | 0.6 | (?) | (?) | 0.4 | (?) | 0.0 | 0.0 | ..... | ..... | ..... |
| *Aspergillus flavus* (series) | 108 | .4 | .6 | .9 | ..... | ..... | ..... | .1— | .4 | .9 | (?) | .5 | .8 | (?) | .4— | 1.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | ABC 3538 | .5 | .8 | 1.0 | ..... | ..... | ..... | .1— | .5 | 1.0 | (?) | .5 | .8 | (?) | (?) | .5— | (?) | 0.0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | Rg 136 | .4 | .9 | 1.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0.1 | .6 | 1.0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | Sc 171 | .4 | .7 | .9 | ..... | ..... | ..... | ..... | ..... | ..... | (?) | .0 | .0 | (?) | .1y | .3— | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Aspergillus fumigatus* | 118 | .6 | 1.0 | 1.0 | ..... | ..... | ..... | ..... | ..... | ..... | .1+ | .9 | 1.0 | .2 | .9 | 1.0 | .2+ | .9 | 1.0 | (?) | .3 | 1.0 | (?) | 0.2b | 0.6b |
| *Aspergillus globosus* | 2705 | .2 | .4 | .7 | (?) | 0.2 | 0.4 | (?) | .2— | .3 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3512 | .2 | .5 | .8 | 0.2 | .4 | .7 | (?) | .2 | .5 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3555.21 | .2 | .5 | .8 | (?) | .4 | .6 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Aspergillus nidulans* | ..... | .3 | .9 | 1.0 | ..... | ..... | ..... | .2+ | .8 | 1.0 | .1— | .4 | .7 | (?) | .3y | .5 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Aspergillus niger* (series) | 111 | .4 | 1.0 | 1.0 | ..... | ..... | ..... | .1+ | .8 | 1.0 | (?) | .5 | 1.0 | (?) | .6 | 1.0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Aspergillus niger*, var. *altipes* | 3534a | .4 | .8 | 1.0 | ..... | ..... | ..... | .2+ | .7 | 1.0 | .1+ | .7 | 1.0 | (?) | (?) | .4 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Aspergillus niger* (*cinnamomeus*) | 3534b | .3 | .7 | 1.0 | ..... | ..... | ..... | .1— | .4 | .6 | (?) | .3— | .8 | (?) | (?) | .3 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Aspergillus niger* (*fuscus*) | 3534c | .3 | .7 | 1.0 | ..... | ..... | ..... | .1 | .4 | .8 | (?) | .3+ | .8 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Aspergillus ochraceus* | 112 | .3 | .7 | 1.0 | ..... | ..... | ..... | (?) | .3 | .7 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0* | (?) | .0 | .0* | (,) | .0 | .0 |
| *Aspergillus oryzae* | 113 | .4 | .6 | .9 | ..... | ..... | ..... | (?) | .3— | .6 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Aspergillus repens* | ..... | .2 | .5 | .8 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .1 | .5 | .6 | (?) | .5 | .8 | 0.1 | .5 | .8 | (?) | .4 | .6 |
| *Aspergillus wentii* | 116 | .2 | .7 | .9 | ..... | ..... | ..... | (?) | .2— | .6 | (?) | .0 | .0 | ..... | ..... | ..... | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Aspergillus* sp | Ra 42 | .3 | .6 | 1.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .2 | .6 | 1.0 | (?) | .4 | 1.0 | (?) | .0 | .0 | (?) | .0 | .0 |
| Do | 3522.30 | .4 | .8 | 1.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | (?) | .0 | .0 | (?) | ..... | ..... | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 3522.36 | .2 | .3 | .5 | ..... | ..... | ..... | .1— | .25 | .5 | (?) | .2— | .3 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 3556 | .3 | .4 | .7 | ..... | ..... | ..... | .1 | .2 | .7 | (?) | .0 | .0 | (?) | (?) | .2y | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Circenella* sp | 3514 Cl. | .3 | .7 | 1.0 | ..... | ..... | ..... | (?) | .5 | .8 | (?) | .2 | .7 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Mucor* sp | 3513 | .3 | .7 | 1.0 | (*) | (*) | (*) | (?) | .2 | 1.0 | ..... | ..... | ..... | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 3523.6 | .4 | 1.0 | 1.0 | ..... | ..... | ..... | .1— | .6 | 1.0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 3560 | .3 | .7 | 1.0 | .1y | .4 | 1.0 | .1y | .7 | 1.0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Rhizopus nigricans* | 3514 D4 | .7 | 1.0 | 1.0 | ..... | ..... | ..... | .4+ | 1.0 | 1.0 | .2+ | 1.0 | 1.0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Syncephalastrum* sp | ..... | .7 | 1.0 | 1.0 | (*) | (*) | (*) | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Fusarium* sp | ..... | .6 | .8 | 1.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .4 | .9 | 1.0 | .2— | .7 | 1.0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Oidium lactis* | ..... | .3 | .7 | .9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .2 | .5 | .9 | (?) | .5 | .9 | (?) | .0 | .0 | (?) | .0 | .0 |
| *Penicillium atramentosum* | 38 | .3 | .5 | .7 | .2 | .4 | .7 | .1 | .2 | .5 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 |
| *Penicillium brevicaule* | 2 | .2 | .6 | 1.0 | .2 | .3 | .8 | .1— | .3 | .7 | (?) | .6+ | .8 | (?) | .4+ | .7 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| | | .3 | .6 | 1.0 | .2 | .4 | .8 | (?) | .2 | .5 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 |

[1] 1.0, a typical spore-bearing colony; 0.1, discernible germination of conidia; tenths, 0.1 to 1.0, relative amount of growth; ?, doubtful; 0, no growth; y, growth of a single spore; —, growth of a few widely scattered spores; +, growth of many spores; *, inharmonious results at times, but usually as given in the table.

TABLE I. —*Comparative effect of heating mold spores in milk to temperatures of from 120° to 150° F. (48.9° to 65.6 C.) for 30 minutes*—Continued

| Name of mold. | Serial No. | Growth of spores when not heated (control). | | | Growth of spores when heated to temperature indicated and held for 30 minutes. | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 120° F. (48.9° C.). | | | 125° F. (51.7° C.). | | | 130° F. (54.5° C.). | | | 135° F. (57.2° C.). | | | 140° F. (60.0° C.). | | | 145° F. (62.8° C.). | | | 150° F. (65.6° C.). | | |
| | | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. | 2 days. | 4 days. | 6 days. |
| *Penicillium camemberti* | 5 | 0.2 | 0.3 | 0.5 | 0.1 | 0.3 | 0.6 | (?) | 0.2y | 0.3 | (?) | 0.0 | 0.0 | (?) | 0.0 | 0.0 | (?) | 0.0 | 0.0 | (?) | 0.0 | 0.0 | ..... | ..... | ..... |
| *Penicillium camemberti*, var. *rogri* | 6 | .2 | .4 | .7 | .2 | .4 | .8 | (?) | .2 | .6 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium chrysogenum* | 26 | .3 | .7 | 1.0 | .3 | .7 | 1.0 | 0.1— | .4 | .8 | (?) | (?) | .2— | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium citrinum* | 15 | .2 | .5 | .8 | (?) | .0 | .0 | (?) | .2— | .4 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium commune* | 23 | .2 | .4 | .8 | ..... | ..... | ..... | .1 | .4 | .6 | (?) | .2— | .3 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium cyclopium* | 2543a | .2 | .5 | .8 | (?) | .2y | .4 | (z) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium divaricatum* | 34 | .3 | .4 | .6 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0.15 | .3+ | .4 | (?) | .2— | .3 | (?) | .0 | .0 |
| *Penicillium expansum* | 14 | .2 | .5 | .7 | (?) | .3 | .8 | (?) | .2 | .6 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium* (*Citromyces*) | 3523.4 | .3 | .5 | .6 | ..... | ..... | ..... | .1 | .4 | .5 | (?) | .3 | .5 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium granulatum* | 9 | .2 | .6 | .8 | ..... | ..... | ..... | .1 | .3 | .6 | (?) | .1y | .2 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium luteum* | 11 | .2 | .4 | .7 | ..... | ..... | ..... | .1— | .3 | .4 | (?) | .3— | .4— | (?) | (?) | .3 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium notatum* | 102 | .2 | .5 | 1.0 | .1 | .4 | .9 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium pinophilum* | 1 | .2 | .5 | 1.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium purpurogenum* | 17 | .3 | .5 | .8 | ..... | ..... | ..... | (?) | .2 | .5 | (?) | .1y | .2 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium roqueforti* | 18 | .2 | .5 | .8 | ..... | ..... | ..... | .1 | .4 | .8 | (?) | .3— | .6 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium rugulosum* | 46 | .2 | .3 | .7 | ..... | ..... | ..... | .1 | .3 | .6 | (?) | .2— | .2 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium solitum* | 2546 | .2 | .5 | .7 | .2 | .4 | .8 | (?) | .3 | .4 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium spinulosum* | 45 | .2 | .4 | .5 | ..... | ..... | ..... | .1— | .3 | .4 | (?) | .2y | .3 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium variabile* | 3551 | .2 | .5 | .8 | .2 | .4 | .9 | (?) | .2— | .4 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| *Penicillium viridicatum* | 2552 | .1 | .2 | .4 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 2643 | .2 | .4 | .7 | .1 | .4 | .8 | (z) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3028 | .3 | .6 | .9 | (?) | .3 | .8 | (?) | .1y | .2 | (?) | .1y | .3 | (?) | .0 | .0 | (!) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3514 A2 | .2 | .4 | .8 | .2 | .6 | .9 | .1 | .5 | 1.0 | ..... | ..... | ..... | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium asperulum* | 2683 | .3 | .5 | .8 | (?) | (?) | .3+ | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 28 | .2 | .3 | .5 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 63 | .3 | .6 | .9 | .1 | .3 | .3 | (?) | .2y | .3 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 103 | .4 | .8 | 1.0 | ..... | ..... | ..... | .3 | 1.0 | 1.0 | 0.1— | .5 | 1.0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 2670 | .2 | .5 | .9 | ..... | ..... | ..... | ..... | ..... | ..... | .1 | .4 | .8 | (?) | .2— | .6 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 3525.61 | .2 | .5 | .8 | ..... | ..... | ..... | .1+ | .3 | .6 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 3553 | .4 | .7 | 1.0 | ..... | ..... | ..... | .15+ | .6 | .9 | (?) | .2— | .5 | (?) | .3— | .6 | (?) | .2 | .8 | (?) | .0 | .0 | ..... | ..... | ..... |
| Do | 3555.18 | .2 | .4 | .7 | .1+ | .4 | .8 | .1 | .5 | .9 | (?) | .0 | .0 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3555.19 | ..... | ..... | ..... | ..... | ..... | ..... | .1 | .9 | 1.0 | (?) | .3 | 1.0 | (?) | (?) | .3 | (?) | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... |

A study of Table I shows that very few mold spores survive exposure to 140° F. (60° C.) in milk for 30 minutes and that at 145° F. (62.8° C.) still fewer are found. With reference to significant organisms, among the mucors the *Mucor racemosus* group (3513, 3523.6, 3560) and *Rhizopus nigricans*, which are found more frequently than all others of this group combined, were destroyed at 130° F. (54.5° C.). The common green species of Penicillium are mostly dead at 130° F. (54.5° C.); a few stand 135° F. (57.2° C.), but two, one of them an undescribed soil organism, survived 140° F. (60° C.) for 30 minutes. Among species of Aspergillus, however, the strains of *A. flavus*, *A. fumigatus*, and *A. repens* all survived 145° F. (62.8° C.) for 30 minutes; *A. repens* and *A. fumigatus* both survived 150° F. (65.6° C.). These three species are always found in forage and feeding stuffs; hence, milk is more or less subject to contamination with them. *A. repens* grows very poorly in milk, however, and the examination of a great many cultures of milk and its products has shown that the actual development of *A. flavus* and *A. fumigatus* is comparatively rare. Although these organisms grow at blood heat and have demonstrated their pathogenicity even to human beings at rare intervals as causes of disease in the lungs, there is no report of their growth in the alimentary canal.

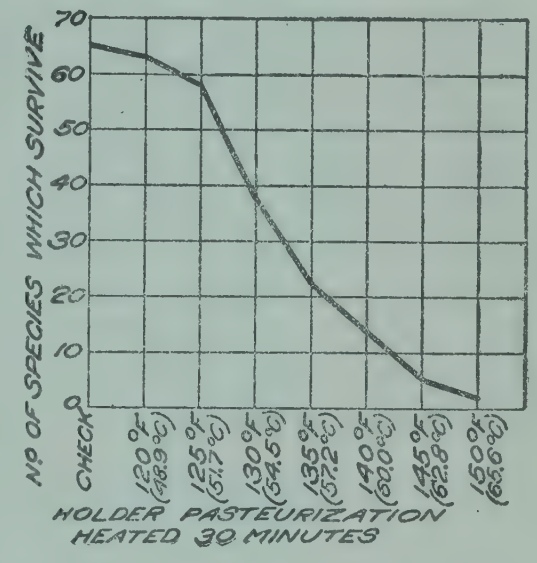


FIG. 1.—Curve of the number of species of molds surviving pasteurization of milk for 30 minutes at a series of temperatures.

The destruction of mold spores by the holder process of pasteurization is shown more clearly in figure 1, where the results have been plotted.

Pasteurization of milk at 145° F. (62.8° C.) may therefore be regarded as destroying mold spores completely enough to render them a negligible factor in the further changes found in the milk.

## EXPERIMENTS WITH THE FLASH PROCESS OF PASTEURIZATION

In working with continuous pasteurizers, temperatures of 165° to 175° F. (73.9° to 79.5° C.) are reached by heating within a period of approximately 30 seconds and maintained about 30 seconds. This is followed by quick cooling. Lower temperatures have not been deemed satisfactory. A series of experiments was therefore planned to subject the freshly inoculated spores of species of Penicillium, Aspergillus, and of the mucors to these temperatures and to determine their relative ability to survive such heating. For this purpose glass tubing about 3 mm. in diameter was drawn into capillary form so that each tube had 3 or 4 inches of the original tub-

ing with 2 to 4 inches of capillary tube approximately 0.5 mm. in diameter. The open end of each tube was plugged with cotton. The tubes were packed into a copper case and dry-sterilized. For each experiment a few drops of sterile milk were transferred to the conidial surface of a colony and the conidia stirred into the milk. A column of milk 15 to 30 mm. long, bearing numerous conidia, was then drawn into the capillary tube and the end sealed in the flame. Experiments had shown that alcohol boiling at 172.4° F. (78° C.) when so treated would boil in 20 to 30 seconds when the tubes were thrust into water at 174.4° F. (79.1° C.). This showed that milk containing mold spores could be heated in from 20 to 30 seconds in capillary tubes to any given temperature when immersed in water 2 degrees Fahrenheit above the desired pasteurizing temperature. In our experiments, therefore, it was possible to duplicate flash pasteurization on a laboratory scale; for example, to pasteurize at 165° F. (73.9° C.) the capillary tubes containing milk and mold spores were held in water at 167° F. (75° C.) for 1 minute. During this period about 30 seconds were required to heat the milk and it was held at the pasteurizing temperature the other half minute. This is approximately the heating period of milk in commercial flash pasteurization. After heating for the required time, the tubes were cooled by thrusting them into cold water. The tip of the capillary was then broken off and the contents streaked upon slanted Czapek's solution agar. The slants were incubated, observed occasionally, and the results of the various experiments were tabulated separately and then brought together in Table II.

TABLE II.—*Comparative effect of heating mold spores in milk to temperatures of from 145° to 175° F. (62.8° to 79.5° C.) for 30 seconds* [1]

| Name of mold. | Serial No. | Growth of spores. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Not heated (control). | | Heated to 145° F. (62.8° C.). | | Not heated (check). | | Heated to 155° F. (68.3° C.). | | Not heated (check). | | Heated to 165° F. (73.9° C.). | | Heated to 175° F. (79.5° C.). | |
| | | 6 days. | 10 days. | 6 days. | 10 days. | 3 days. | 6 days. | 3 days. | 6 days. | 4 days. | 6 days. | 4 days. | 6 days. | 4 days. | 6 days. |
| *Aspergillus candidus* | 106 | 0.4 | 0.7 | 0.8 | 1.0 | 0.3 | 0.7 | 0.0 | 0.0 | .... | .... | .... | .... | .... | .... |
| *Aspergillus flavus* series | 108 | .9 | 1.0 | .8 | 1.0 | .4 | .8 | .0 | .0 | 0.6 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Do | 3538. 108 | .9 | 1.0 | .6 | 1.0 | .4 | .8 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| Do | Rg136 | .8 | 1.0 | .6 | 1.0 | .5 | .8 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| Do | Sc171 | .7 | .9 | .0 | .0 | .5 | .9 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Aspergillus fumigatus* | 118 | .9 | 1.0 | .9 | 1.0 | .3 | .8 | .0 | .0 | .6 | 1.0 | .0 | .0 | .0 | .0 |
| *Aspergillus globosus?* | 2705 | .8 | 1.0 | .0 | .0 | .... | .... | .0 | .0 | .4 | .8 | .5? | 1.0? | .0 | .0 |
| Do | 3512 | .... | .... | .... | .... | .3 | .7 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| Do | 3555. 21 | .8 | .9 | .0 | .0 | .3 | .6 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Aspergillus nidulans* | 110 | .3 | 1.0 | .3 | 1.0 | .3 | .8 | .0 | .0 | .5 | .9 | .0 | .0 | .0 | .0 |
| *Aspergillus niger* | 111 | .9 | 1.0 | .0 | .0 | .3 | .7 | .0 | .0 | .6 | 1.0 | .0 | .0 | .0 | .0 |
| *Aspergillus niger*, var. *altipes* | 3534-a | .9 | 1.0 | .6 | 1.0 | .5 | .8 | ? | .4? | .... | .... | .... | .... | .... | .... |
| *Aspergillus cinnamomeus* | 3534-b | .8 | 1.0 | .6 | 1.0 | .5 | 1.0 | .0 | .0 | .5 | 1.0 | .0 | .0 | .0 | .0 |
| *Aspergillus fuscus* | 3534-c | .8 | 1.0 | .7 | .9 | .... | .... | .0 | .0 | .7 | 1.0 | .0 | .0 | .0 | .0 |
| *Aspergillus ochraceus* | 112 | .9 | 1.0 | .0 | .0 | .9 | 1.0 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Aspergillus oryzae* | 113 | .8 | 1.0 | .0 | .0 | .9 | 1.0 | .0 | .0 | .5 | .8 | .0 | .0 | .0 | .0 |

[1] 1.0, a typical colony; decimals, proportionate growth; 0.0, no growth; ?, inharmonious results.

TABLE II.—*Comparative effect of heating mold spores in milk to temperatures of from 145° to 175° F. (62.8° to 79.5° C.) for 30 seconds*—Continued

| Name of mold. | Serial No. | Growth of spores. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Not heated (control). | | Heated to 145° F. (62.8° C.). | | Not heated (check). | | Heated to 155° F. (68.3° C.). | | Not heated (check). | | Heated to 165° F. (73.9° C.). | | Heated to 175° F. (79.5° C.). | |
| | | 6 days. | 10 days. | 6 days. | 10 days. | 3 days. | 6 days. | 3 days. | 6 days. | 4 days. | 6 days. | 4 days. | 6 days. | 4 days. | 6 days. |
| *Aspergillus repens* | ..... | .... | .... | .... | .... | 0.8 | 1.0 | 0.8 | 1.0 | .... | .... | .... | .... | .... | .... |
| *Aspergillus wentii* | 116 | .8 | 1.0 | .0 | .0 | .5 | 1.0 | ? | .5? | .... | .... | .... | .... | .... | .... |
| *Aspergillus* sp | Ra42 | .7 | 1.0 | .6 | 1.0 | .8 | 1.0 | .0 | .0 | .5 | .8 | .0 | .0 | .0 | .0 |
| Do | 3522.30 | .9 | 1.0 | .0 | .0 | .9 | 1.0 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| Do | 3522.36 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| Do | 3556 | .9 | 1.0 | .0 | .0 | .5 | 1.0 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Aspergillus parasiticus* | 3509 | .9 | 1.0 | .6 | 1.0 | .5 | 1.0 | ? | .6? | .7 | 1.0 | .0 | .0 | .0 | .0 |
| Do | 3565 | .9 | 1.0 | .5 | 1.0 | .7 | .9 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Circinella umbellata* | 3514. Cl | .8 | 1.0 | .8 | 1.0 | .5 | 1.0 | .0 | .0 | .8 | 1.0 | .0 | .0 | .0 | .0 |
| *Mucor racemosus* (group) | 3513 | .8 | .8 | .8 | 1.0 | .9 | 1.0 | .0 | .0 | .7 | 1.0 | .0 | .0 | .0 | .0 |
| Do | 3523.6 | .... | .... | .... | .... | .9 | 1.0 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| Do | 3560 | 1.0 | 1.0 | 1.0 | 1.0 | .... | .... | .0 | .0 | .9 | 1.0 | .0 | .0 | .0 | .0 |
| *Rhizopus nigricans* | 3Rn. | .8 | 1.0 | .0 | .0 | .6 | 1.0 | .0 | .0 | .7 | 1.0 | .0 | .0 | .0 | .0 |
| *Syncephalastrum* sp | Syn. | .9 | 1.0 | .8 | 1.0 | .... | .... | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Fusarium* sp | ..... | .8 | 1.0 | .9 | 1.0 | .5 | 1.0 | ? | .7 | .... | .... | .... | .... | .... | .... |
| *Penicillium atramentosum* | 38 | .8 | 1.0 | 9.0 | 1.0 | .... | .... | .... | .... | .5 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium biforme* | 39 | .9 | 1.0 | .0 | .0 | .3 | .6 | .0 | .0 | .5 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium brevicaule* | 2 | .3 | bact. | .0 | .0 | .3 | .6 | .0 | .0 | .5 | .6 | .0 | .0 | .0 | .0 |
| *Penicillium camemberti* | 5 | .9 | 1.0 | .0 | .0 | .9 | 1.0 | .0 | .0 | .3 | .7 | .0 | .0 | .0 | .0 |
| *Penicillium camemberti*, var. *rogeri* | 6 | .8 | 1.0 | .0 | .0 | .4 | .6 | .0 | .0 | .4 | .9 | .0 | .0 | .0 | .0 |
| *Penicillium chrysogenum* | 26 | .8 | 1.0 | .0 | .0 | .4 | .6 | .0 | .0 | .0 | .0 | .0 | .0 | .0 | .0 |
| *Penicillium citrinum* | 15 | .7 | 1.0 | .6 | .7 | .... | .... | .0 | .0 | .9 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium commune* | 23 | .8 | 1.0 | .6 | .8 | .9 | 1.0 | .0 | .0 | .7 | 1.0 | .6? | 1.0 | .0 | .0 |
| *Penicillium cyclopium* | 2543-a | .8 | 1.0 | .4 | .8 | .5 | .6 | .0 | .0 | .5 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium digitatum* | 16 | .6 | 1.0 | .0 | .0 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| *Penicillium divaricatum* | 34 | .9 | 1.0 | .9 | 1.0 | .4 | .6 | .0 | .0 | .8 | .9 | .0 | .0 | .0 | .0 |
| *Penicillium duclauxi* | 20 | .8 | 1.0 | .4 | .5 | .5 | .9 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Penicillium expansum* | 14 | .... | .... | .... | .... | .5 | .9 | .0 | .0 | .6 | .8 | .0 | .0 | .2? | .5? |
| *Penicillium* (*Citromyces*) sp. | 3523.4 | .9 | 1.0 | .0 | 1.0 | .5 | .9 | .0 | .0 | .4 | .8 | .0 | .0 | .0 | .0 |
| *Penicillium granulatum* | 9 | .9 | 1.0 | .0 | .0 | .5 | .9 | .0 | .0 | .4 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium italicum* | 10 | .4 | .9 | .5 | 1.0 | .3 | .6 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Penicillium luteum* | 11 | .8 | .9 | .4 | 1.0 | .4 | .8 | Very | slow. | .5 | .9 | ? | ? | .0 | .0 |
| *Penicillium notatum* | 102 | .6 | .7 | .1 | .5? | .3 | .8 | .0 | .0 | .3 | .7 | .0 | .0 | .0 | .0 |
| *Penicillium oxalicum* | 103 | .8 | .9 | ? | .7? | .5 | .8 | .4 | .8 | .9 | .0 | .0 | .0 | .0 | .0 |
| *Penicillium pinophilum* | 1 | .4 | .8 | .4 | .8 | .4 | .9 | .0 | .0 | .3 | .7 | .0 | .0 | .0 | .0 |
| *Penicillium puberulum*? | 2683 | .8 | 1.0 | .0 | .0 | .4 | .9 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Penicillium purpurogenum* | 17 | .9 | 1.0 | .0 | .0 | .3 | .7 | .0 | .0 | .6 | .9 | .0 | .0 | .0 | .0 |
| *Penicillium purpurogenum*, var. *rubri sclerotium* | 2670 | .... | .... | .... | .... | .3 | .6 | .0 | .0 | .5 | 1.0 | .3 | .5 | .0 | .6? |
| *Penicillium roqueforti* | 18 | .... | .... | .... | .... | .4 | .8 | .0 | .0 | .5 | .9 | .0 | .0 | .0 | .0 |
| *Penicillium rugulosum* | 46 | .4 | .8 | .4 | .8 | .... | .... | .... | .... | .3 | .4 | .0 | .0 | .0 | .0 |
| *Penicillium solitum* | 2546 | .8 | 1.0 | .0 | .0 | .3 | .6 | .0 | .0 | .8 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium solitum*? | 66 | .9 | 1.0 | .6 | 1.0 | .5 | .9 | .0 | .0 | .5 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium spinulosum* | 45 | .8 | 1.0 | ? | .5 | .... | .... | .... | .... | .5 | .8 | .0 | .0 | .0 | .0 |
| *Penicillium stoloniferum* | 27 | .9 | 1.0 | .4? | 1.0? | .4 | .9 | .0 | .0 | .5 | .8 | .0 | .0 | .0 | .0 |
| *Penicillium variabile* | 3551 | .9 | 1.0 | .0 | .0 | .3 | 1.0 | ? | .7 | .8 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium viridicatum* | 2552 | .9 | 1.0 | ? | .6 | .3 | .9 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| *Penicillium viridicatum*, var.? | 2643 | .9 | 1.0 | .7 | .8 | .3 | .7 | .0 | .0 | .5 | .7 | .0 | .0 | .0 | ? |
| Do | 3028 | .7 | 1.0 | .0 | .0 | .3 | .8 | .0 | .0 | .7 | 1.0 | .8? | .9? | .0 | .0 |
| Do | 3514-a | .8 | 1.0 | .4? | .6? | .4 | .6 | .0 | .0 | .5 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium* (*Citromyces*) sp. | 28 | .... | .... | .6 | .8 | .3 | .6 | .0 | .0 | .4 | .9 | .0 | .0 | .0 | .0 |
| Do | 63 | .9 | 1.0 | .0 | .0 | .4 | .7 | .0 | .0 | .5 | 1.0 | .0 | .0 | .0 | .0 |
| *Penicillium* sp | 3525.61 | .8 | 1.0 | ? | .4 | .4 | .8 | .0 | .0 | .... | .... | .... | .... | .... | .... |
| Do | 3553 | .... | .... | .... | .... | .5 | .9 | .0 | .0 | .6 | .9 | .0 | .0 | .0 | .0 |
| Do | 3555.18 | .... | .... | .... | .... | .... | .... | .... | .... | .4 | .7 | .0 | .0 | .0 | .0 |
| Do | 3555.19 | .7 | .9 | .4 | .7 | .6 | .9 | .0 | .0 | .... | .... | .... | .... | .... | .... |

From Table II it is seen that very few of the forms are killed in 30 seconds at 145° F. (62.8° C.); nearly all, however, are destroyed at 155° F. (68.3° C.). None of the colonies found at 165° F. (73.9° C.) and 175° F. (79.5° C.) were produced in both tubes. The chance of error is not fully eliminated in these cases. The consistent character of the whole table and the innocuous character of the few organisms in which occasional colonies occurred after heating show that temperatures of 165° to 175° F. (73.9° to 79.5° C.) for 30 seconds do practically destroy the spores of these molds as they may be found in milk, although a few conidia in some species may occasionally survive.

Figure 2 shows graphically the effect of the flash process of pasteurization on mold spores.

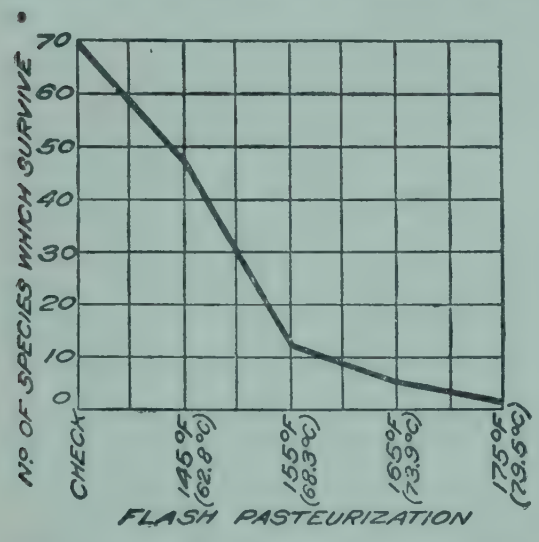


FIG. 2.—Curve of the number of species of molds surviving flash pasteurization at a series of temperatures.

## DESTRUCTION OF MOLD SPORES BY DRY HEAT

The third series of experiments was planned to find the relative ability of the spores of approximately the same organisms to endure heating in dry air for the same period as used for heating in milk. After some experimentation the following method was used: Strips of heavy filter paper were cut wide enough so that only the edges would come into contact with the glass when dropped into test tubes. A drop of sterile water carrying a suspension of the spores under experiment was deposited in the middle of the paper strip and allowed to evaporate overnight. The tubes were then immersed in liquid heated to the desired temperature and held 30 minutes after check tubes carrying thermometers indicated that the air in the tubes had reached the same degree. The tubes were then removed and cooled. Melted agar was allowed to run into each tube to form a slant and the cultures were set away at room temperature. Observations of growth were made as in the previous experiments and the results tabulated in the same manner in Table III.

TABLE III.—*Comparative ability of mold spores to survive heating in dry air for 30 minutes at temperatures of 180° to 250° F. (82.2° to 121.1° C.)*[1]

| Name of mold. | Serial No. | Growth of spores when not heated (control) and after having been heated to the temperature indicated for 30 minutes. | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Not heated (control) 5 days. | Heated to 180° F. (82.2° C.) 5 days. | Not heated (control) 7 days. | Heated to 190° F. (87.8° C.). | | Not heated (control). | | Heated to 200° F. (93.3° C.). | | Not heated (control). | | Heated to 210° F. (98.9° C.). | | Heated to 220° F. (104.5° C.). | | Not heated (control). | | Heated to 230° F. (110.0° C.). | | Not heated (control). | | Heated to 250° F. (121.1° C.). | |
| | | | | | 3 days. | 7 days. | 4 days. | 8 days. | 4 days. | 8 days. | 4 days. | 7 days. | 4 days. | 7 days. | 4 days. | 7 days. | 3 days. | 6 days. | 3 days. | 6 days. | 4 days. | 8 days. | 4 days. | 8 days. |
| *Aspergillus candidus* | 106 | 0.5 | 0.3 | 0.4 | ..... | 0.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ? | 0.6 | ? | ?0.0 | ? | 0.4 | 0.0 | 0.0 |
| *Aspergillus flavus*, var | 108 | .7 | .7 | ..... | ..... | ..... | 0.3 | ..... | ..... | 0.1 | 0.7 | ..... | 0.2 | 0.9 | ..... | ..... | 0.2 | .9 | 0.3 | ..... | 0.5 | .8 | .0 | .0 |
| Do | 3538.108 | .5 | .5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .5 | ..... | ? | .5 | ..... | ..... | .3 | .9 | .5 | 1.0 | .5 | .8 | ? | .6? |
| Do | Rg136 | .8 | .6 | .8 | ..... | .3 | .4 | ..... | ..... | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | Sc171 | .5 | .4 | ..... | ..... | ..... | .4 | ..... | 0.3 | .4 | .4 | ..... | ..... | ..... | ..... | ..... | .2 | .9 | .0 | .0 | ..... | ..... | ..... | ..... |
| *Aspergillus fumigatus* | 118 | .8 | .6 | ..... | ..... | ..... | .3 | ..... | ? | .6 | .5 | ..... | .3 | .8 | 0.3 | ..... | .2 | .9 | .3 | 1.0 | .5 | .9 | .0 | .0 |
| *Aspergillus globosus* | 2705 | .6 | .4 | ..... | ..... | ..... | .2 | ..... | ? | .2 | .8 | ..... | .0 | .0 | ..... | ..... | .2 | .8 | .0 | .0 | .3 | .7 | .0 | .0 |
| *Aspergillus globosus* ? | 3512 | .8 | .4 | .8 | ..... | .0 | .4 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ? | .9 | .2 | 1.0 | .4 | .9 | .0 | .0 |
| *Aspergillus versicolor* ? | 3555.21 | .5 | .5 | .8 | ..... | .0 | .3 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | .2 | .9 | ? | ? | .3 | .6 | .0 | .0 |
| *Aspergillus nidulans* | 110 | .5 | .5 | ..... | ..... | ..... | .2 | ..... | ? | .4 | .6 | ..... | .0 | .0 | ..... | ..... | .3 | .9 | ? | ? | .4 | .8 | .0 | .0 |
| *Aspergillus niger* | 111 | .7 | .5 | ..... | ..... | ..... | .4 | ..... | ? | .3 | .8 | ..... | .0 | .0 | ..... | ..... | .3 | .9 | .5 | .8 | ..... | ..... | ..... | ..... |
| *Aspergillus niger*, var. *altipes* | 3534a | .8 | .6 | ..... | ..... | ..... | .3 | ..... | ? | .5 | ..... | ..... | ..... | ..... | ..... | ..... | .2 | 1.0 | .6 | 1.0 | .4 | .8 | .0 | .0 |
| *Aspergillus cinnamomeus* | 3534b | .7 | .3 | ..... | ..... | ..... | .3 | ..... | ? | .4 | .8 | ..... | ? | ..... | .3 | ..... | .2 | .9 | .6 | .8 | .4 | .7 | .0 | .0 |
| *Aspergillus fuscus* | 3534c | .8 | ..... | ..... | ..... | ..... | .5 | ..... | ? | .4 | .7 | ..... | .0 | .0 | ..... | ..... | .2 | .9 | .5 | 1.0 | .4 | .7 | .0 | .0 |
| *Aspergillus ochraceus* | 112 | .7 | .4 | .8 | ..... | .0 | .4 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | .3 | .9 | ? | ? | ..... | ..... | ..... | ..... |
| *Aspergillus oryzae* | 113 | .8 | .5 | ..... | ..... | ..... | .3 | ..... | ? | .4 | .8 | ..... | .0 | .0 | .2 | ..... | .3 | .7 | .0 | .0 | ..... | ..... | ..... | ..... |
| *Aspergillus repens* | ..... | .4 | .2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .4 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | .2 | .4 | .0 | .0 |
| *Aspergillus wentii* | 116 | .7 | .0 | .7 | ..... | .0 | .5 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Aspergillus* sp | Ra42 | .6 | .3 | .5 | ..... | .3 | .2 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | .2 | .8 | .0 | .0 | ..... | ..... | ..... | ..... |
| Do | 3522.30 | .8 | .4 | ..... | ..... | ..... | .2 | ..... | .4 | .5 | .7 | ..... | .0 | .0 | ..... | ..... | .2 | .8 | ? | .3 | .3 | .6 | .0 | .0 |
| Do | 3522.36 | .5 | .2 | .5 | ..... | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3556 | .9 | .6 | ..... | ..... | ..... | .4 | ..... | ? | .6 | .8 | ..... | .0 | .0 | ..... | ..... | .3 | .9 | .3 | .5 | .4 | .8 | .0 | .0 |
| *Aspergillus parasiticus* | 3509 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .3 | .9 | .3 | 1.0 | .4 | .7 | .0 | .0 |
| *Aspergillus* sp | 3565 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .3 | .9 | .3 | 1.0 | .4 | .7 | .0 | .0 |
| *Circinella umbellata* | 3514 C1 | ..... | ..... | ..... | ..... | ..... | .3 | ..... | ? | .4 | .6 | ..... | ? | .6 | .3 | 0.7 | .5 | .9 | .5 | .8 | .3 | .7 | ? | .4 |
| *Mucor racemosus* ? | 3513 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .4 | ..... | .4 | .8 | ..... | .5 | .4 | .9 | .7 | 1.0 | .4 | .7 | ? | .8 |
| Do | 3523.6 | .6 | .5 | ..... | ..... | ..... | .3 | ..... | .3 | 1.0 | .6 | ..... | ? | .7 | ..... | ..... | .4 | .9 | .9 | .8 | .4 | .8 | .0 | .0 |
| Do | 3560 | .7 | ..... | ..... | ..... | ..... | .3 | ..... | .4 | .4 | .8 | 0.9 | ? | .9 | .8 | .8 | .5 | 1.0 | .9 | .9 | .6 | 1.0 | ? | 1.0 |
| *Rhizopus nigricans* | ..... | .6 | .6 | ..... | ..... | ..... | .3 | 0.5 | .3 | .5 | .3 | .9 | .3 | .9 | .3 | .7 | .3 | .8 | .6 | .9 | .3 | .6 | .0 | .0 |
| *Syncephalastrum* sp | ..... | .5 | .5 | ..... | ..... | ..... | .2 | ..... | ? | .4 | ? | .9 | ? | .6 | ? | ? | .2 | .9 | ? | .5 | ..... | ..... | ..... | ..... |
| *Fusarium* sp | ..... | .7 | .5 | ..... | ..... | ..... | .5 | .8 | .4 | .8 | ..... | .7 | .5 | .8 | ..... | ..... | .2 | .8 | ? | .6 | .4 | .6 | .0 | .0 |

[1] 1.0, a typical colony: decimals, proportionate growth, 0.0, no growth; ? inharmonious results: y, growth of a single spore,

TABLE III.—*Comparative ability of mold spores to survive heating in dry air for 30 minutes at temperatures of 180° to 250° F. (82.2° to 121.1° C.)*—Contd.

| Name of mold. | Serial No. | Growth of spores when not heated (control) and after having been heated to the temperature indicated for 30 minutes. | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Not heated (control) 5 days. | Heated to 180° F. (82.2° C.) 5 days. | Not heated (control) 7 days. | Heated to 190° F. (87.8° C.), | | Not heated (control). | | Heated to 200° F. (93.3° C.). | | Not heated (control). | | Heated to 210° F. (98.9° C.). | | Heated to 220° F. (104.5° C.). | | Not heated (control). | | Heated to 230° F. (110.0° C.). | | Not heated (control). | | Heated to 250° F. (121.1° C.). | |
| | | | | | 3 days. | 7 days. | 4 days. | 8 days. | 4 days. | 8 days. | 4 days. | 7 days. | 4 days. | 7 days. | 4 days. | 7 days. | 3 days. | 6 days. | 3 days. | 6 days. | 4 days. | 8 days. | 4 days. | 8 days. |
| *Penicillium atramentosum* | 38 | 0.7 | 0.5 | 0.9 | 0.3 | 0.6 | 0.4 | ..... | 0.0 | 0.0 | ..... | ..... | ..... | ..... | ..... | ..... | ? | 0.8 | 0.0 | 0.0 | ..... | ..... | ..... | ..... |
| *Penicillium biforme* | 37 | ..... | ..... | ..... | ..... | ..... | .6 | ..... | .0 | .0 | 0.7 | ..... | 0.0 | 0.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium brevicaule* | 2 | .8 | .0 | .7 | .0 | .0 | .4 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium camemberti* | 5 | .7 | .3 | .8 | .0 | .0 | .5 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium camemberti* var. *rogeri* | 6 | .6 | .3 | .8 | ? | .2 | .7 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium chrysogenum* | 26 | .9 | .8 | ..... | ..... | ..... | .7 | ..... | ? | .7? | .8 | ..... | .4 | .6 | ..... | ..... | .3 | .9 | .4 | 1.0 | .5 | .9 | .0 | .0 |
| *Penicillium citrinum* | 15 | .8 | .0 | .9 | .0 | .0 | .5 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium commune* | 23 | .8 | .4 | .7 | .0 | .0 | .6 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium cyclopium* | 2543a | .8 | ..... | ..... | ..... | ..... | ? | ..... | ? | .2 | .8 | ..... | .0 | .0 | ..... | ..... | .2 | 1.0 | ? | ? | .4 | .9 | .0 | .0 |
| *Penicillium digitatum* | 16 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium divaricatum* | 34 | .7 | .6 | ..... | ..... | ..... | .3 | ..... | ? | .6 | .8 | ..... | ? | .3 | ..... | ..... | .2 | 1.0 | .7 | 1.0 | .3 | .7 | .0 | .0 |
| *Penicillium duclauxi* | 20 | .6 | .5 | ..... | ..... | ..... | .3 | ..... | ? | .2 | .5 | ..... | .4 | .7 | ..... | ..... | .3 | .9 | ? | ? | ..... | ..... | ..... | ..... |
| *Penicillium expansum* | 14 | ..... | ..... | .7 | ? | .5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium glabrum* (*Citromyces*) | 3523.4Y | .9 | .6 | .9 | ? | .2 | .5 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium granulatum* | 9 | .7 | ..... | .9 | .0 | .0 | .5 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium italicum* | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .3 | .9 | .0 | .0 | ..... | ..... | ..... | ..... |
| *Penicillium luteum* | 11 | .5 | .4 | .3 | ? | .3 | .3 | ..... | ? | .4 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium notatum* | 102 | .8 | .6 | .7 | ? | .4 | .6 | ..... | ? | 1.0 | .8 | ..... | .4 | .8 | .0 | .0 | .2 | .9 | ? | ? | ..... | ..... | ..... | ..... |
| *Penicillium oxalicum* | 103 | .8 | .7 | ..... | ..... | ..... | .7 | ..... | ? | .4 | .8 | ..... | .4? | .8 | ..... | ..... | ..... | ..... | ..... | ..... | .4 | .8 | .0 | .0 |
| *Penicillium pinophilum* | 1 | .5 | .4 | ..... | ..... | ..... | .2 | ..... | ? | .2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium puberulum* | 2683 | ..... | ..... | .8 | ? | .6 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium purpurogenum* | 17 | .7 | .3 | .7 | .1 | .4 | .3 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium purpurogenum*, var. *rubri-sclerotium* | 2670 | ..... | ..... | .6 | .4 | .9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium roqueforti* | 18 | .7 | .4 | .6 | .0 | .0 | .4 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium rugulosum* | 46 | .5 | .3 | .8 | ? | .4 | .3 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium solitum* | 2546 | .7 | .4 | .8 | .0 | .0 | .5 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium solitum* ? | 66 | ..... | ..... | ..... | ..... | ..... | .6 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium spinulosum* | 45 | .5 | .3 | ..... | ..... | ..... | .5 | ..... | ? | .3? | .7 | ..... | .1 | .5 | ..... | ..... | .3 | .9 | .6 | 1.0 | .8 | .8 | .0 | .0 |
| *Penicillium stoloniferum* | 27 | ..... | ..... | .8 | .0 | .0 | .7 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium variabile* | 3551 | .7 | .5 | ..... | ..... | ..... | .5 | ..... | ? | .3 | .7 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Penicillium viridicatum* | 2552 | .9 | .4 | ..... | ? | ..... | .5 | ..... | ? | .4? | .8 | ..... | ? | .6 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium viridicatum*, var. ? | 2643 | .8 | .3 | .8 | ? | .3 | .6 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3028 | .8 | .3 | .7 | .0 | .0 | .5 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3514 $A_2$ | .9 | .5 | .9 | ? | .3 | .6 | ..... | .0 | .0 | .8 | ..... | .3 | .8 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 40008 | ..... | ..... | ..... | .5 | .4 | .5 | ..... | ? | .4 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium* (*Citromyces*) sp. | 28 | .5 | .2 | .8 | .0 | .0 | .4 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 63 | .9 | .5 | .9 | .0 | .0 | .7 | ..... | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *Penicillium* sp. | 3525.61 | ..... | ..... | .8 | ? | .4 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | .2 | ..... | .9 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3553 | 1.0 | ..... | ..... | ..... | ..... | .6 | ..... | ? | .2 | .6 | ..... | .4 | .5 | ..... | .9 | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3555.18 | .8 | .5 | .8 | ? | .3 | .5 | ..... | .0 | .0 | ..... | ..... | ..... | .3 | ..... | .9 | .0 | .0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Do | 3555.19 | ..... | ..... | ..... | ..... | ..... | .8 | ..... | ? | .3 | .8 | ..... | .1 | .6 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

A study of Table III shows that mold spores possess much greater ability to withstand dry heat than heating in milk. Very few forms were destroyed at 180° F. (82.2° C.), but they include *Penicillium brevicaule*, which has a thick-walled spore and in laboratory cultures has remained viable at least 7 years. Only a few species of Penicillium survived heating to 200° F. (93.3° C.) for 30 minutes. All these are forms which grew at 98.6° F. (37° C.), and some of them are widely distributed.

Aside from *A. wentii*, all the species of Aspergillus survived heating at 200° F. (93.3° C.). Several of them survived at 230° F. (110° C.), but after 250° F. (121.1° C.) for 30 minutes no species showed growth after 6 days' incubation. Three of six mucors, however, survived the heating to 250° F. (121.1° C.) for 30 minutes. These species were killed quickly by both forms of heating in milk. The results of these experiments are plotted in figure 3.

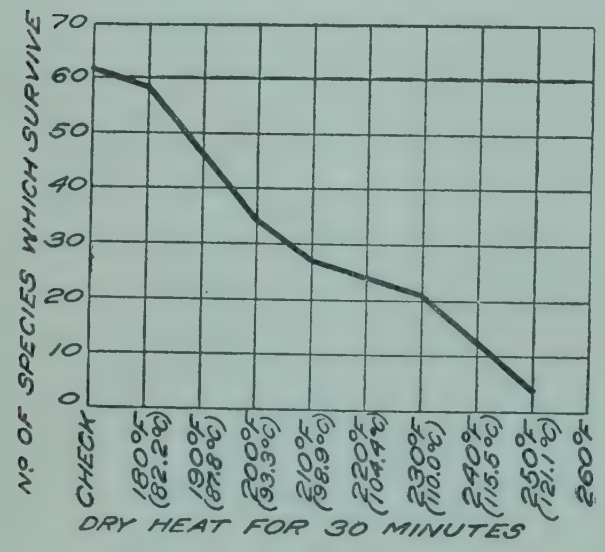


FIG. 3.—Curve of the number of species of molds surviving dry heat for 30 minutes at a series of temperatures.

The destruction of mold spores by dry heat has no relation to the subject of pasteurization of milk, but it is of scientific interest.

## DISCUSSION OF RESULTS

These results with mold spores agree in general with bacteriological studies of pasteurization. Very few of these organisms found in milk survive after 30 minutes' heating to 145° F. (62.8° C.). Certain molds, notably *Aspergillus fumigatus* and *A. flavus*, do survive, but they occur only occasionally in milk. *Oidium lactis* and the mucors, which are probably more important as milk-borne organisms than all the rest, are destroyed at the low temperatures used in the holder process of pasteurization. In the flash process very few mold spores survived at 165° F. (73.9° C.). Occasionally some spores seem to have escaped destruction at 175° F. (79.5° C.), but the organisms surviving in these cases were of minor importance in the decomposition of dairy products. In confirmation of these results the writers have had access to unpublished data of Mr. R. O. Webster, of the Bureau of Chemistry, giving cultural analysis of butter made from flash-pasteurized cream on a commercial basis. Cultures from this butter showed no mold spores, while cultures made at the same time from country butter showed 20,000 to 60,000 per gram.

Mold spores in milk seem, therefore, to be destroyed completely or reduced to negligible numbers by both of the standard pasteurization processes.

Careful study of the cultures showed that the first effect of heating was to delay germination. This is indicated in the tables by the reports of successive examinations of the same culture. In Table I three reports are given; later only two reports. The third and fourth observations, however, were usually made. At times heating to a degree just under the death point delayed germination almost the full length of the usual growing period of the species. The number of possible sources of error was so great that the results of observations have been tabulated and compared. When essential harmony of results was not obtained, the work was repeated. In a few cases the continued lack of consistent results for particular organisms is indicated by the interrogation point in the tables. Even with these precautions the data obtained can be said to apply only to the strains used. This is indicated by comparing the results given for the *Aspergillus flavus* group or for the four members of the *A. niger* group. These results do not prove that other strains of these groups would respond exactly as here tabulated. In fact, more extended studies (as yet unpublished) of these two groups indicate that organisms otherwise undistinguishable may differ greatly if we measure a single physiological reaction. Such quantitative differences may persist in continued cultures, but are hardly comparable to differences in the kind of reaction as a basis for separating species. Inside the race or strain, conidia transferred from the same culture respond very differently. There is frequently a survival of a few spores where a majority of the spores die. There may be, therefore, a difference of as much as 20° F. (11.1 C.) between the temperature at which an occasional culture is completely killed and that at which cultures of that species are uniformly killed. These results resemble those obtained in determining the thermal death point of bacteria.

The applicability of these results to the occurrence of mold spores in substances other than milk has not been tested. The variation in composition of the substratum together with the heating may at times introduce a considerable variation. In general, however, it is clear that mold spores are easily killed by heat when suspended in fluid. The tables have been studied in an attempt to correlate resistance with size of spore or thickness of spore wall. No such correlation has been found. There is, therefore, no suggestion as to the nature of the difference in these organisms which affects their resistance to heat.

## SUMMARY

(1) The holder process of pasteurization, in which milk was heated to 145° F. (62.8° C.) and maintained at that temperature for 30 minutes, killed the conidia of every species investigated, except those of *Asper-*

*gillus repens*, *A. flavus*, and *A. fumigatus*. The molds which survive are found only occasionally in milk.

(2) The flash process of pasteurization, where milk was heated to 165° F. (73.9° C.) for a period of 30 seconds, destroyed the spores of all the molds tested with the exception of many spores of one form and occasional spores of three more forms. At 175° F. (79.5° C.) only occasional spores of two forms developed.

(3) When the heating process was performed in dry air for a period of 30 seconds at 200° F. (93.3° C.), 31 out of 42 forms of Penicillium and 7 out of 24 forms of Aspergillus were destroyed, but none of the cultures of the mucors. A temperature of 250° F. (121.1° C.) over a period of 30 minutes killed all the forms of *Penicillium* spp. tried, but left an occasional living spore in one species of Aspergillus and three out of six mucors.

# EFFECT OF WATER IN THE RATION ON THE COMPOSITION OF MILK

By W. F. Turner, R. H. Shaw, R. P. Norton, and P. A. Wright, *of the Dairy Division, Bureau of Animal Industry*

## INTRODUCTION

Experiments conducted at Brownsville, Tex., by the Dairy Division of the Bureau of Animal Industry indicate that the feeding of prickly-pear (*Opuntia* spp.) lowers the percentage of fat in milk. In comparison with other feeds prickly-pear contains a large amount of water and mineral matter. It was thought by the writers that one or both of these constituents might be responsible for the reduction in fat percentage; consequently experiments were conducted at Beltsville, Md., to determine the influence of the water. Work with the mineral matter is now in progress.

The literature dealing with the effects of watery feeds or water in the ration upon the quantity and the quality of milk produced contains many conflicting statements. No doubt the difficulty of eliminating all factors except the watery character of the ration is largely responsible for the conflicting nature of these statements.

Gilchrist (1)[1] reports very little difference, if any, in quantity and quality between the milk produced by cows either on pasture only or on a daily ration of mangels in varying amounts up to 86 pounds per cow and that produced by the same cows on a ration of hay and grain.

Tangl and Zaitschek (12) state, as the result of extensive experiments to determine the influence of watery feeds on milk secretion, that there is no difference between the composition of the milk from cows fed on a watery ration and that from cows fed on a dry one. They state that it is not true that watery feeds cause the production of thinner milk than dry feeds.

Lauder and Fagan (10, p. 9) reached the following conclusions from experiments extending over a 3-year period, using 60 cows and feeding a large ration of turnips (*Brassica rapa*) to compare with a dry or concentrated ration:

> The feeding of a ration containing a large quantity of water does not increase the percentage of water in the milk or reduce the percentage of fat.
>
> The greater yield of milk was obtained from the cows on the concentrated ration. On the other hand, the milk from the cows on the turnip ration contained a higher percentage of fat, and a greater total weight of fat was secreted in the milk.

[1] Reference is made by number to "Literature cited," p. 177–178.

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Dept. of Agriculture, Washington, D. C. Apr. 24, 1916
di A—21

Holtsmark (6) reports that there is no decrease in the fat content of the milk of cows on a liberal daily ration of concentrated feed and cut straw, with as much as 77 pounds of turnips per head, after this ration is substituted for one consisting of hay, straw, concentrates, and a small quantity of roots.

A writer in the Journal of the Board of Agriculture (3), London, England, concludes from a study of the work of various investigators that, although many feeds have a specific effect on the yield and quality of milk, it may be attributed to stimulating substances in the feeds rather than to water content. These substances have a physiological rather than a nutritive effect and are present in feeds in small quantities only.

As the result of a number of experiments conducted and a review of previous work of the same character, Jordan (8, p. 69) states that, "Contrary to a notion held by many, it is not possible to water a cow's milk through her drink or through the ingesting of watery feed."

The Journal of the Board of Agriculture, London (2), reports that a dairyman was convicted in the French courts for selling adulterated milk. The conviction was based upon the assumption that it is possible to water milk either by feeding cows on watery feeds, by causing them to drink water in large quantities, or by making them drink immediately before milking. To prove the fallacy of this assumption, the Board conducted experiments with a number of cows. After feeding them an excess of common salt (sodium chlorid), or limiting the water drunk after free access to it, or permitting them to drink only immediately before milking, it was found that no change is produced in the composition of the milk.

At Offerton Hall, Durham, England, a series of experiments was conducted to determine how the composition of milk is affected by feeding wet brewers' grains. The first of these experiments (7, p. 35) indicates that the feeding of these grains to cows whose milk is habitually low in butter fat is not to be recommended, especially during the earlier stages of the lactation period, when the grains tend slightly to reduce the yield of fat. The writer advises dairymen to use such grains sparingly. Later experiments (13, p. 19–20) indicate that the grains may be fed safely if the ration contains other feeds also, and that there is no appreciable lowering of the butter fat when the grains are fed in moderate quantities.

In a general article upon the effect of different feeds upon the quality of milk, McConnell (11) says:

> It is a matter of common knowledge that the lush grass of spring, an excess of mangolds, or too many brewers' grains will promote a great flow of milk, but that that milk will be poor, and farmers who do not do anything to modify such feeding will find their milk coming dangerously near the "standard."

Hansson (4), of the Stockholm Agricultural Experiment Station, in a review of the work of various investigators concerning the effect of different feeds upon the fat content of milk, concludes that there are on

this point distinct differences among different feeds, but that the effect of any feed depends upon the composition of the other components of the ration. He states that roots have a favorable effect upon milk secretion, but tend slightly to lower the fat content.

Koch (9) reports extensive feeding experiments at Rosenhof in which cows were fed beet roots (*Beta vulgaris*), and gives the following conclusions:

> An increase in fat units (total fat) with beet-root feed, an increase of the amount of milk combined with a decrease in the fat content. However, the increase in quantity exceeded the decrease in quality so much that the cows gave 6 per cent more total fat on the beet-root ration.[1]

## PLAN OF INVESTIGATION

The experimental work to determine the effect of water in the ration upon the composition of milk was conducted at the Dairy Division farm, Beltsville, Md., and included parts of three different lactation periods. The four following methods for supplying rations of widely different water content were tried:

1. A full allowance of drinking water as compared with a limited supply, the ration otherwise being alike in both cases.
2. A heavy ration of turnips as compared with a dry-roughage one.
3. Wet beet pulp as compared with dry beet pulp.
4. Green crimson clover (*Trifolium incarnatum*) as compared with the cured hay.

As the change in the fat content of the milk noted during the prickly-pear experiments took place within a few days after the change in the character of the ration and continued throughout the 80-day period, it was decided that for this work two 10-day periods of feeding any one ration, with a 10-day transition period intervening, and equal periods of feeding the comparative ration, would give time enough for any change in the composition of the milk to take place. In each series of experiments the milk from each cow was weighed at each milking, and 10-day composite samples were taken for analysis. The data obtained from each series of experiments are given separately.

## FULL VERSUS LIMITED ALLOWANCE OF WATER

In this series of experiments eight cows were used and all received the same general treatment. For the first two 10-day periods the animals were given water ad libitum twice daily. Then a definite quantity of water, not more than 75 per cent of the full allowance, and in some cases less than 65 per cent, was given for two 10-day periods following a 10-day transition period. The quantity of water given in the limited water ration was so reduced that, when watered once a day, all cows drank the quantity allowed. After a second 10-day transition period, a full allowance of water was again given for two 10-day periods. This completed the work

[1] Authors' translation.

with all but two cows, which were given a still more reduced allowance of water following the second full-allowance period. Table I gives the results for each cow.

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*

COW 100

| Water allowance. | Total milk. | Total water. | Fat. | | Specific gravity. | Solids not fat. | Moisture. | Ash. | Total protein. |
|---|---|---|---|---|---|---|---|---|---|
| | *Pounds.* | *Pounds.* | *Per cent.* | *Pounds.* | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| Full | 220.6 | 412.5 | 4.50 | 9.93 | 1.033 | 9.11 | 86.39 | 0.720 | 3.35 |
| Do. | 240.6 | 502.0 | 4.50 | 10.83 | 1.032 | 9.19 | 86.31 | .710 | 3.35 |
| Transition | 205.3 | 340.0 | 4.60 | 9.44 | 1.033 | 9.27 | 86.13 | .720 | 3.36 |
| Limited | 198.8 | 340.0 | 4.80 | 9.54 | 1.034 | 9.36 | 85.84 | .710 | 3.52 |
| Do. | 199.3 | 340.0 | 4.70 | 9.37 | 1.033 | 9.18 | 86.12 | .705 | 3.53 |
| Transition | 197.6 | 434.0 | 4.35 | 8.60 | 1.032 | 9.11 | 86.54 | .700 | 3.41 |
| Full | 172.2 | 378.0 | 4.90 | 8.44 | 1.032 | 9.01 | 86.09 | .745 | 3.68 |
| Do. | 167.0 | 358.0 | 4.70 | 7.85 | 1.033 | 9.18 | 86.12 | .747 | 3.66 |
| Transition | 149.8 | 200.0 | 4.88 | 7.19 | 1.033 | 9.36 | 85.76 | .755 | 3.80 |
| Limited | 135.8 | 205.0 | 4.90 | 5.65 | 1.032 | 9.30 | 85.80 | .770 | 3.72 |
| Do. | 138.0 | 215.0 | 4.80 | 6.62 | 1.032 | 9.13 | 86.07 | .750 | 3.61 |
| Average: | | | | | | | | | |
| Full | 200.1 | 412.5 | 4.65 | 9.26 | ..... | 9.12 | 86.23 | .730 | 3.51 |
| Limited | 168.0 | 275.0 | 4.80 | 7.79 | ..... | 9.24 | 85.96 | .734 | 3.59 |

COW 21

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Full | 191.8 | 500.5 | 6.00 | 11.51 | 1.036 | 9.98 | 84.02 | .770 | 3.92 |
| Do. | 188.0 | 502.0 | 6.00 | 10.88 | 1.035 | 10.14 | 83.86 | .770 | 3.94 |
| Transition | 179.0 | 320.0 | 6.25 | 11.19 | 1.036 | 10.18 | 83.57 | .750 | 3.94 |
| Limited | 175.2 | 320.0 | 6.10 | 10.69 | 1.035 | 10.11 | 83.79 | .740 | 3.87 |
| Do. | 179.0 | 320.0 | 6.03 | 10.79 | 1.035 | 10.21 | 83.76 | .740 | 3.96 |
| Transition | 181.6 | 496.0 | 5.50 | 9.99 | 1.034 | 9.91 | 84.59 | .730 | 3.82 |
| Full | 184.5 | 462.0 | 5.73 | 10.57 | 1.035 | 10.00 | 84.27 | .720 | 3.90 |
| Do. | 180.4 | 473.0 | 6.00 | 10.82 | 1.035 | 9.81 | 84.19 | .740 | 3.99 |
| Transition | 172.9 | 250.0 | 5.95 | 10.29 | 1.035 | 9.95 | 84.07 | .755 | 3.91 |
| Limited | 157.8 | 255.0 | 6.00 | 9.47 | 1.034 | 10.10 | 83.90 | .750 | 3.91 |
| Do. | 151.5 | 280.0 | 5.80 | 8.79 | 1.033 | 9.82 | 84.38 | .730 | 3.76 |
| Average: | | | | | | | | | |
| Full | 186.2 | 484.0 | 5.93 | 10.94 | ..... | 9.98 | 84.08 | .750 | 3.94 |
| Limited | 165.9 | 294.0 | 5.98 | 9.94 | ..... | 10.06 | 83.96 | .740 | 3.88 |

COW 19

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Full | 220.5 | 520.0 | 5.30 | 11.69 | 1.036 | 9.83 | 84.87 | .770 | 3.73 |
| Do. | 228.6 | 492.0 | 5.20 | 11.89 | 1.035 | 9.82 | 84.98 | .745 | 3.86 |
| Transition | 213.0 | 300.0 | 5.18 | 11.03 | 1.036 | 10.09 | 84.73 | .735 | 3.73 |
| Limited | 213.1 | 345.0 | 5.42 | 11.55 | 1.035 | 9.66 | 84.92 | .750 | 3.77 |
| Do. | 202.5 | 305.0 | 5.33 | 10.79 | 1.035 | 9.98 | 84.69 | .780 | 3.82 |
| Transition | 203.3 | 622.0 | 5.60 | 11.38 | 1.034 | 9.66 | 84.74 | .765 | 3.83 |
| Full | 198.5 | 520.0 | 5.50 | 10.92 | 1.034 | 9.74 | 84.76 | .775 | 3.88 |
| Do. | 193.2 | 520.0 | 5.25 | 10.14 | 1.034 | 9.71 | 85.04 | .765 | 3.74 |
| Average: | | | | | | | | | |
| Full | 205.2 | 513.0 | 5.31 | 11.16 | ..... | 9.77 | 84.91 | .764 | 3.80 |
| Limited | 207.8 | 325.0 | 5.37 | 11.17 | ..... | 9.82 | 84.80 | .765 | 3.79 |

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*—Continued

COW 8

| Water allowance. | Total milk. | Total water. | Fat. | | Specific gravity. | Solids not fat. | Moisture. | Ash. | Total protein. |
|---|---|---|---|---|---|---|---|---|---|
| | *Pounds.* | *Pounds.* | *Per cent.* | *Pounds.* | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent* |
| Full | 252.2 | 573.0 | 4.55 | 11.48 | 1.033 | 9.30 | 86.15 | 0.730 | 3.17 |
| Do | 253.0 | 553.0 | 4.15 | 10.50 | 1.033 | 9.10 | 86.75 | .723 | 3.12 |
| Transition | 234.9 | 346.0 | 4.20 | 10.46 | 1.034 | 9.39 | 86.41 | .707 | 3.20 |
| Limited | 220.7 | 350.0 | 4.30 | 9.49 | 1.034 | 9.44 | 86.26 | .731 | 3.14 |
| Do | 208.0 | 350.0 | 4.65 | 9.67 | 1.034 | 9.35 | 86.00 | .714 | 3.11 |
| Transition | 217.6 | 509.0 | 4.30 | 9.37 | 1.033 | 8.98 | 86.72 | .727 | 3.11 |
| Full | 205.7 | 500.0 | 4.30 | 8.85 | 1.034 | 9.25 | 86.45 | .724 | 3.06 |
| Do | 209.4 | 536.0 | 4.50 | 9.42 | 1.033 | 9.18 | 86.32 | .732 | 3.17 |
| Average: | | | | | | | | | |
| Full | 230.1 | 540.0 | 4.37 | 10.06 | ..... | 9.21 | 86.88 | .727 | 3.13 |
| Limited | 214.3 | 350.0 | 4.47 | 9.58 | ..... | 9.39 | 86.13 | .722 | 3.12 |

COW 17

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Full | 194.9 | 465.0 | 5.30 | 10.33 | 1.034 | 9.73 | 84.97 | .74 | 3.70 |
| Do | 206.8 | 437.0 | 4.95 | 10.24 | 1.035 | 9.88 | 85.17 | .72 | 3.77 |
| Transition | 173.5 | 267.0 | 5.28 | 9.02 | 1.035 | 9.73 | 84.99 | .715 | 3.62 |
| Limited | 188.2 | 310.0 | 5.15 | 9.69 | 1.034 | 9.53 | 85.32 | .720 | 3.68 |
| Do | 174.0 | 300.0 | 5.20 | 9.05 | 1.035 | 9.92 | 84.88 | .755 | 3.68 |
| Transition | 184.7 | 443.0 | 5.18 | 9.57 | 1.034 | 9.44 | 85.38 | .740 | 3.81 |
| Full | 164.9 | 499.0 | 5.20 | 8.57 | 1.033 | 9.69 | 85.11 | .770 | 3.86 |
| Do | 156.4 | 504.0 | 5.18 | 8.10 | 1.033 | 9.70 | 85.12 | .755 | 3.76 |
| Average: | | | | | | | | | |
| Full | 180.7 | 476.0 | 5.16 | 9.31 | ..... | 9.75 | 85.09 | .744 | 3.77 |
| Limited | 181.1 | 305.0 | 5.17 | 9.37 | ..... | 9.72 | 85.10 | .737 | 3.68 |

COW 9

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Full | 199.7 | 410.0 | 4.40 | 8.79 | 1.031 | 8.83 | 86.77 | .744 | 2.78 |
| Do | 193.7 | 432.0 | 4.15 | 8.04 | 1.030 | 8.40 | 87.45 | .709 | 2.65 |
| Transition | 181.0 | 386.0 | 4.20 | 7.60 | 1.031 | 8.53 | 87.27 | .711 | 2.76 |
| Limited | 163.5 | 300.0 | 4.05 | 6.62 | 1.032 | 8.79 | 87.16 | .724 | 2.72 |
| Do | 142.9 | 300.0 | 4.15 | 5.93 | 1.031 | 8.64 | 87.21 | .703 | 2.54 |
| Transition | 165.7 | 526.0 | 4.10 | 6.79 | 1.030 | 8.27 | 87.63 | .698 | 2.69 |
| Full | 170.4 | 556.0 | 4.15 | 7.07 | 1.031 | 8.53 | 87.32 | .712 | 2.73 |
| Do | 164.9 | 541.0 | 4.30 | 7.09 | 1.031 | 8.65 | 87.05 | .704 | 2.84 |
| Average: | | | | | | | | | |
| Full | 182.2 | 485.0 | 4.25 | 7.75 | ..... | 8.60 | 87.15 | .717 | 2.75 |
| Limited | 153.2 | 300.0 | 4.10 | 5.77 | ..... | 8.71 | 87.18 | .713 | 2.63 |

COW 14

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Full | 269.1 | 429.0 | 5.10 | 13.72 | 1.033 | 9.17 | 85.73 | .723 | 3.04 |
| Do | 263.9 | 470.0 | 4.80 | 12.67 | 1.032 | 9.11 | 86.09 | .723 | 3.10 |
| Transition | 236.5 | 338.0 | 5.40 | 12.77 | 1.033 | 9.10 | 85.50 | .754 | 3.18 |
| Limited | 232.9 | 305.0 | 5.00 | 11.64 | 1.033 | 8.97 | 86.03 | .756 | 3.22 |
| Do | 222.6 | 300.0 | 5.10 | 11.36 | 1.033 | 8.91 | 85.99 | .739 | 3.25 |
| Transition | 235.2 | 566.0 | 4.90 | 11.52 | 1.032 | 8.84 | 86.26 | .727 | 3.16 |
| Full | 227.6 | 494.0 | 5.05 | 11.50 | 1.032 | 9.06 | 85.89 | .737 | 3.21 |
| Do | 211.1 | 484.0 | 4.70 | 9.92 | 1.031 | 8.99 | 86.31 | .724 | 3.06 |
| Average: | | | | | | | | | |
| Full | 242.9 | 469.0 | 4.91 | 11.95 | ..... | 9.09 | 86.00 | .727 | 3.10 |
| Limited | 227.7 | 302.0 | 5.05 | 11.50 | ..... | 8.94 | 86.51 | .747 | 3.23 |

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*—Continued

COW 2

| Water allowance. | Total milk. | Total water. | Fat. | | Specific gravity. | Solids not fat. | Moisture. | Ash. | Total protein. |
|---|---|---|---|---|---|---|---|---|---|
| | *Pounds.* | *Pounds.* | *Per cent.* | *Pounds.* | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent* |
| Full | 238.6 | 517.0 | 5.60 | 13.37 | 1.033 | 9.55 | 84.85 | 0.724 | 3.50 |
| Do | 236.5 | 595.0 | 4.85 | 11.47 | 1.034 | 9.68 | 85.47 | .737 | 3.67 |
| Transition | 201.6 | 388.0 | 5.60 | 11.29 | 1.035 | 9.92 | 84.48 | .775 | 3.85 |
| Limited | 181.9 | 350.0 | 5.50 | 10.00 | 1.035 | 9.57 | 84.93 | .769 | 3.82 |
| Do | 188.5 | 350.0 | 5.55 | 10.47 | 1.035 | 9.57 | 84.88 | .746 | 3.77 |
| Transition | 172.7 | 589.0 | 5.30 | 9.15 | 1.032 | 9.32 | 85.38 | .738 | 3.48 |
| Full | 189.9 | 630.0 | 4.95 | 9.40 | 1.034 | 9.54 | 85.51 | .754 | 3.56 |
| Do | 202.9 | 639.0 | 4.70 | 9.54 | 1.033 | 9.62 | 85.68 | .747 | 3.89 |
| Average: | | | | | | | | | |
| Full | 217.0 | 595.0 | 5.02 | 10.94 | ..... | 9.60 | 85.38 | .740 | 3.65 |
| Limited | 185.2 | 350.0 | 5.52 | 10.23 | ..... | 9.57 | 84.90 | .757 | 3.79 |

In studying the data obtained in these trials it will be noted that all the milk constituents except the fat show very little variation during the different periods, and that these differences are attributable more to the individual animals than to the character of the ration. Taking the average figures for the two classes of rations, it will be seen that the full water allowance ration tended to increase the quantity of milk produced and to cause a slight reduction of the fat content of the milk. A study of the data for individual cows by separate periods, however, will show that this average effect of the different rations is caused more by the order in which the rations are fed than by their character. Of the data obtained from the eight cows used in this test those from only one (No. 2) show indication of any effect of the ration upon the composition of the milk, and the data from the seven other cows are so negative that this variation is probably caused more by the individual than by the ration. Two of the cows, Nos. 17 and 19, show practically no variation in either quantity or quality of the milk produced; one other, No. 100, decreased gradually in the quantity of milk produced and increased gradually in quality, regardless of the ration; while the remaining four, Nos. 8, 9, 14, and 21, gave milk the fat content of which varied considerably from normal in different periods, even on the same ration. These variations were independent of the character of the ration—that is, the abnormal percentage of fat was in some cases found when the full allowance of water was given and in other cases when the quantity was reduced. A summing up of all the data obtained shows that the feeding of rations whose water content is varied by controlling the quantity of water drunk has no influence upon the composition of the milk produced.

## TURNIPS VERSUS DRY-ROUGHAGE RATION

In this series of experiments four cows were used, the experimental period consisting of six test periods and two transition periods. Figure 1 shows the grouping of the cows and the character of the ration fed during each period.

As much as 90 pounds of turnips a day was fed to the cows on the wet-roughage ration, with the addition of 4 pounds of clover hay. The roughage ration of the dry-roughage group consisted entirely of clover hay. The grain ration was the same for both groups. In Table II

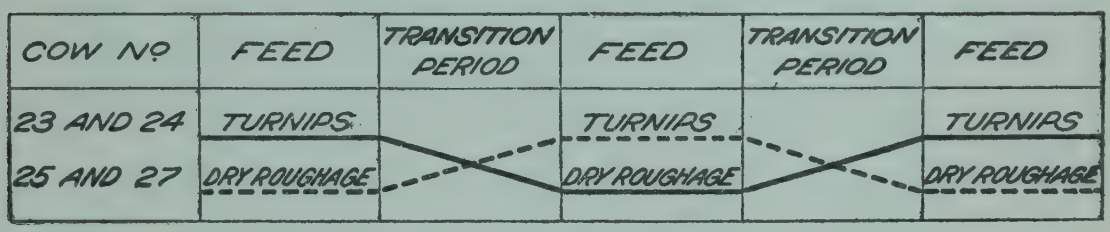


FIG. 1.—Grouping of cows and kind of ration fed cows 23, 24, 25, and 27

both the quantity of water drunk and the total water content of the turnips are given, turnips being considered as having 90 per cent of water, as shown by Henry and Morrison (5, p. 645).

TABLE II.—*Comparison of the effect of turnips and a dry-roughage ration on the composition of milk*

COW 23

| Ration. | Total milk. | Water in ration. | Turnips. | Fat. | | Specific gravity. | Solids not fat. | Moisture. | Ash. | Total protein. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Lb.* | *Lb.* | *Lb.* | *P. ct.* | *Lb.* | | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* |
| Wet | 234.7 | 94 | 774 | 4.10 | 9.62 | 1.032 | 8.64 | 87.26 | 0.750 | 3.18 |
| Do | 236.9 | 123 | 810 | 4.30 | 10.19 | 1.030 | 8.50 | 87.20 | .720 | 3.09 |
| Transition | 225.2 | 446 | 261 | 4.03 | 9.08 | 1.031 | 8.52 | 87.45 | .735 | 3.17 |
| Dry | 212.4 | 712 | ..... | 4.00 | 8.50 | 1.030 | 8.44 | 87.56 | .715 | 3.09 |
| Do | 214.6 | 734 | ..... | 4.03 | 8.65 | 1.030 | 8.40 | 87.57 | .700 | 3.13 |
| Transition | 204.2 | 190 | 630 | 3.90 | 7.96 | 1.030 | 8.55 | 87.55 | .725 | 3.11 |
| Wet | 198.6 | 62 | 810 | 4.13 | 8.20 | 1.030 | 8.47 | 87.40 | .725 | 3.24 |
| Do | 192.9 | 88 | 810 | 4.05 | 7.81 | 1.031 | 8.69 | 87.26 | .740 | 3.28 |
| Average: | | | | | | | | | | |
| Wet | 215.8 | 92 | 801 | 4.14 | 8.95 | ..... | 8.57 | 87.28 | .732 | 3.20 |
| Dry | 213.5 | 723 | ..... | 4.01 | 8.57 | ..... | 8.42 | 87.56 | .707 | 3.11 |

COW 24

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Wet | 255.1 | 84 | 774 | 4.10 | 10.50 | 1.035 | 9.56 | 86.34 | .710 | 3.50 |
| Do | 251.3 | 72 | 810 | 4.10 | 10.30 | 1.035 | 9.73 | 86.17 | .690 | 3.55 |
| Transition | 234.5 | 389 | 261 | 4.30 | 10.08 | 1.035 | 9.48 | 86.22 | .690 | 3.51 |
| Dry | 226.4 | 607 | ..... | 3.80 | 8.60 | 1.034 | 9.32 | 86.88 | .645 | 3.37 |
| Do | 226.7 | 658 | ..... | 4.00 | 9.07 | 1.033 | 9.21 | 86.79 | .635 | 3.47 |
| Transition | 234.0 | 119 | 630 | 3.81 | 8.92 | 1.033 | 9.44 | 86.75 | .695 | 3.44 |
| Wet | 237.2 | 56 | 810 | 4.10 | 9.73 | 1.033 | 9.31 | 86.59 | .680 | 3.51 |
| Do | 227.1 | 144 | 810 | 4.10 | 9.31 | 1.034 | 9.51 | 86.39 | .715 | 3.64 |
| Average: | | | | | | | | | | |
| Wet | 242.7 | 89 | 801 | 4.10 | 9.96 | ..... | 9.53 | 86.37 | .699 | 3.52 |
| Dry | 226.5 | 632 | ..... | 3.90 | 8.83 | ..... | 9.26 | 86.83 | .640 | 3.42 |

TABLE II.—*Comparison of the effect of turnips and a dry-roughage ration on the composition of milk*—Continued

COW 25

| Ration. | Total milk. | Water in ration. | Turnips. | Fat. | | Specific gravity. | Solids not fat. | Moisture. | Ash. | Total protein. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Lb.* | *Lb.* | *Lb.* | *P. ct.* | *Lb.* | | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* |
| Dry | 199.8 | 604 | ..... | 4.23 | 8.45 | 1.033 | 9.11 | 86.66 | .750 | 3.14 |
| Do | 195.5 | 541 | ..... | 4.30 | 8.41 | 1.033 | 9.07 | 86.63 | .730 | 3.11 |
| Transition | 218.2 | 158 | 85 | 4.25 | 9.27 | 1.032 | 8.89 | 86.86 | .745 | 3.21 |
| Wet | 203.0 | ..... | 810 | 3.98 | 8.08 | 1.032 | 9.03 | 86.99 | .715 | 3.34 |
| Do | 198.0 | ..... | 810 | 4.00 | 7.92 | 1.033 | 9.10 | 86.90 | .725 | 3.40 |
| Transition | 177.3 | 304 | 180 | 4.23 | 7.50 | 1.032 | 9.17 | 86.60 | .755 | 3.49 |
| Dry | 175.3 | 505 | ..... | 4.50 | 7.89 | 1.032 | 8.81 | 86.69 | .715 | 3.18 |
| Do | 160.4 | 488 | ..... | 4.55 | 7.30 | 1.031 | 8.87 | 86.58 | .730 | 3.28 |
| Average: | | | | | | | | | | |
| Dry | 182.7 | 532 | ..... | 4.39 | 8.01 | ..... | 9.01 | 86.64 | .731 | 3.18 |
| Wet | 200.0 | ..... | 810 | 3.99 | 8.00 | ..... | 9.06 | 86.94 | .720 | 3.37 |

COW 27

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dry | 223.2 | 591 | ..... | 4.30 | 9.60 | 1.033 | 9.04 | 86.66 | .730 | 3.13 |
| Do | 207.7 | 578 | ..... | 4.10 | 8.52 | 1.033 | 9.18 | 86.72 | .710 | 3.12 |
| Transition | 223.3 | 132 | 585 | 4.20 | 9.38 | 1.032 | 8.90 | 86.89 | .735 | 3.14 |
| Wet | 237.8 | ..... | 810 | 4.00 | 9.51 | 1.033 | 8.81 | 87.19 | .695 | 2.99 |
| Do | 240.0 | ..... | 810 | 4.04 | 9.70 | 1.033 | 9.02 | 86.94 | .765 | 3.29 |
| Transition | 214.0 | 407 | 180 | 4.03 | 8.62 | 1.034 | 9.09 | 86.88 | .745 | 3.24 |
| Dry | 199.0 | 548 | ..... | 4.05 | 8.06 | 1.032 | 9.01 | 86.94 | .750 | 3.28 |
| Do | 175.4 | 588 | ..... | 4.30 | 7.54 | 1.031 | 8.82 | 86.88 | .765 | 3.15 |
| Average: | | | | | | | | | | |
| Dry | 201.3 | 576 | ..... | 4.19 | 8.43 | ..... | 9.01 | 86.80 | .741 | 3.17 |
| Wet | 238.9 | ..... | 810 | 4.02 | 9.60 | ..... | 8.91 | 87.06 | .730 | 3.14 |

In this series of experiments the data show conflicting results. All the cows gave more milk when fed the turnip ration, and they also ate that ration much more readily than they did the entire dry-roughage one. The two cows that were fed the ration in the order wet-dry-wet gave milk of a higher fat content on the wet ration, while those fed in the dry-wet-dry order gave the higher percentage of fat when the dry ration alone was fed. None of the other constituents of the milk were appreciably affected, and in the case of the fat content the data are so conflicting that they seem to have been caused by some factor other than the ration.

## DRY VERSUS WET BEET PULP

Two cows were used in this trial, one being fed wet, dry, and wet beet pulp in successive periods, with a transition period after each change in ration, and the ration of the second cow being just the reverse. While being fed dry beet pulp each cow received 10 pounds daily. The wet ration consisted of 40 pounds of the wet beet pulp, or 10 pounds of the

dry, with 30 pounds of water added, the pulp used having been found to absorb three times its weight of water. In all conditions except as to the pulp the two rations were alike for each cow in the different periods. In Table III the quantity of water in the beet pulp, as well as the quantity of water drunk, is given:

TABLE III.—*Comparison of the effect of dry beet pulp and wet beet pulp on the composition of milk*

COW 22

| Ration. | Total milk. | Water in ration. | Pulp. | Fat. | | Specific gravity. | Solids not fat. | Moisture. | Ash. | Total protein. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Lb.* | *Lb.* | *Lb.* | *Per ct.* | *Lb.* | | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* |
| Dry | 209.6 | 590 | ..... | 4.80 | 10.06 | 1.034 | 9.93 | 85.27 | 0.769 | 3.65 |
| Do | 201.8 | 540 | ..... | 4.85 | 9.79 | 1.036 | 10.08 | 85.07 | .797 | 3.70 |
| Wet | 199.3 | 273 | 300 | 4.65 | 9.27 | 1.035 | 10.00 | 85.35 | .789 | 3.89 |
| Do | 189.5 | 306 | 300 | 4.65 | 8.81 | 1.035 | 10.15 | 85.20 | .796 | 3.88 |
| Transition | 185.4 | 487 | 49 | 4.80 | 8.90 | 1.035 | 9.85 | 85.35 | .795 | 3.92 |
| Dry | 180.9 | 479 | ..... | 4.80 | 8.68 | 1.036 | 9.76 | 85.44 | .797 | 3.88 |
| Do | 167.7 | 472 | ..... | 4.90 | 8.22 | 1.035 | 9.59 | 85.51 | .790 | 3.82 |
| Average: | | | | | | | | | | |
| Dry | 190.0 | 520 | ..... | 4.84 | 9.19 | ..... | 9.84 | 85.32 | .788 | 3.76 |
| Wet | 194.4 | 290 | 300 | 4.65 | 9.04 | ..... | 10.07 | 85.27 | .792 | 3.88 |

COW 18

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Wet | 193.7 | 340 | 300 | 5.10 | 9.88 | 1.032 | 9.23 | 85.67 | .740 | 3.11 |
| Do | 185.0 | 369 | 300 | 5.20 | 9.62 | 1.033 | 9.19 | 85.61 | .747 | 3.30 |
| Dry | 196.6 | 472 | ..... | 5.00 | 9.83 | 1.034 | 9.60 | 85.40 | .730 | 3.51 |
| Do | 176.9 | 511 | ..... | 5.40 | 9.55 | 1.033 | 9.48 | 85.12 | .733 | 3.41 |
| Transition | 183.2 | 348 | 228 | 5.20 | 9.53 | 1.034 | 9.45 | 85.35 | .760 | 3.62 |
| Wet | 166.9 | 327 | 300 | 5.60 | 9.35 | 1.035 | 9.40 | 85.00 | .766 | 3.64 |
| Do | 159.6 | 383 | 300 | 5.60 | 8.94 | 1.034 | 9.30 | 85.10 | .754 | 3.65 |
| Average: | | | | | | | | | | |
| Wet | 176.3 | 355 | 300 | 5.37 | 9.45 | ..... | 9.28 | 85.34 | .752 | 3.42 |
| Dry | 186.7 | 492 | ..... | 5.20 | 9.69 | ..... | 9.54 | 85.27 | .731 | 3.46 |

The data from these two cows give negative results so far as the effect of the water in the ration upon the composition of the milk is concerned. One cow, No. 22, gave milk slightly lower in fat content when the wet beet pulp was fed; but the other gave opposite results, the milk testing higher than that produced when the preceding dry ration was fed. The quantity of milk produced by both cows decreased at a normal rate.

## GREEN VERSUS CURED CRIMSON CLOVER

In this series of experiments four cows were used. For a period of 10 days they were each fed all the fresh-cut green crimson clover that they would consume, and composite samples were taken during the period.

Later, when the clover had been harvested and had become well cured, the same four cows were fed all the cured product that they would consume, and composite samples again taken. No weights of water drunk were taken, but as the green clover contained 71.23 per cent of water and the cured hay but 8.33 per cent, there was an appreciable difference in the quantity of water in the rations of the two test periods. Table IV gives the results for each cow. The figures in parentheses following the class of ration show the total number of pounds of the cured or green clover fed.

TABLE IV.—*Comparison of the effect of green and cured crimson clover on the composition of milk*

| Ration. | Milk. | Total water in roughage. | Fat. | | Specific gravity. | Moisture. | Ash. | Total protein. |
|---|---|---|---|---|---|---|---|---|
| | | | | | COW 23 | | | |
| | *Lb.* | *Lb.* | *Per ct.* | *Lb.* | | *Per ct.* | *Per ct.* | *Per ct.* |
| Green (405) | 132.0 | 288 | 5.81 | 4.40 | 1.029 | 86.94 | 0.723 | 3.18 |
| Cured (180) | 107.1 | 15 | 4.53 | 4.23 | 1.031 | 86.97 | .744 | 3.38 |
| | | | | | COW 25 | | | |
| Green (415) | 163.2 | 296 | 4.05 | 6.61 | 1.030 | 87.26 | .724 | 3.17 |
| Cured (180) | 167.3 | 15 | 3.60 | 6.02 | 1.032 | 87.45 | .742 | 3.19 |
| | | | | | COW 27 | | | |
| Green (400) | 161.1 | 285 | 3.75 | 6.04 | 1.030 | 87.58 | .738 | 3.05 |
| Cured (165) | 128.0 | 14 | 3.60 | 4.61 | 1.032 | 87.35 | .783 | 3.17 |
| | | | | | COW 201 | | | |
| Green (505) | 333.5 | 360 | 3.65 | 12.17 | 1.028 | 88.36 | .696 | 2.78 |
| Cured (220) | 297.2 | 18 | 3.20 | 9.51 | 1.030 | 88.85 | .725 | 2.77 |

The length of time covered by this series of experiments, 10 days on each ration, was too short to give more than an indication of the results which a complete investigation would give. The data obtained, however, show that the water in the ration supplied by a green roughage, as compared with the cured product, does not lower the fat content of the milk. The results of these experiments would even indicate an opposite effect, for in all cases the cows gave higher testing milk and three of them produced more milk on the green feed.

SUMMARY

Four different methods of varying the water content of the ration were used in this work.

(1) A full versus a limited allowance of drinking water.
(2) Turnips versus a dry-roughage ration.
(3) Wet versus dry beet pulp.
(4) Green versus dry crimson clover.

Eight cows were used in the experiments conducted by the first method, four in the second, two in the third, and four in the fourth.

In every case except when the crimson clover was fed the amount of water drunk by the different animals, as well as the difference in the water content of the roughages under comparison, was determined.

With all except one cow, No. 22 in the wet versus dry beet-pulp group, the amount of water in the dry ration did not exceed 75 per cent of that supplied by the wet ration, and with some cows that were given a limited allowance of water the dry ration contained less than 60 per cent of the water content of the full-allowance ration.

Cow 22 in the wet versus dry beet-pulp group received, when the dry ration was fed, 88 per cent of the water content of the wet ration.

In the green versus cured crimson-clover group, the former contained 71.23 per cent water and the latter 8.33 per cent. The daily ration of green clover varied from 40 to 50 pounds per head, and of the cured hay from 16 to 22 pounds per head.

Certain individual cows at times produced milk having an abnormal fat content. This effect was apparently independent of the ration, as it occurred not only with the high water-content ration but with the dry as well. A study of the data obtained in the four series shows that the watery character of the ration has no effect upon the fat content of the milk.

There was even less variation in the other milk constituents than in the fat. This indicates that rations of varying water content have no effect upon the composition of milk.

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# CROWNGALL STUDIES SHOWING CHANGES IN PLANT STRUCTURES DUE TO A CHANGED STIMULUS

[PRELIMINARY PAPER]

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Some recent experiments with crowngall have led to a number of discoveries which may be summarized as follows:

First, as everyone knows, the tendency of cambium is not simply to go on indefinitely producing more cambium but to elaborate out of its embryonic elements formed structures, tracheids, wood vessels, wood fibers, ray cells, sieve tubes, etc., all having a definite arrangement and a well-defined polarity, but when internodal stem cambium is inoculated with the crowngall organism (*Bacterium tumefaciens*) the ordinary physiological tendencies are upset, as already shown in 1911 and 1912,[1] and several very interesting new phenomena make their appearance: (1) The elements of the formed or mature tissues are produced in less numbers than ordinarily, and these elements have lost the whole or a considerable part of their polarity, so that the most bizarre complexes of twisted and distorted tissues arise; (2) the parenchymatous elements are greatly increased in number and reduced in size, since under the bacterial stimulus many of the cambium cells appear to have lost all power to produce mature tissues and at the same time have acquired a new growth impetus, a tendency to an uncontrolled, pathologically embryonic, cell multiplication, the result of which is a tumor of indefinite extension—the ordinary naked crowngall, containing the distorted formed elements above referred to and in addition exhibiting a marked hyperplasia of the parenchyma; (3) correlative with these changes, over which the plant has no control, is a tendency to open wounds and to early decay and also to the formation of daughter tumors.

Second, when, by means of very shallow needle pricks, similar inoculations are made into the internodal cortex of young stems (the so-called fundamental tissue, which is still in a growing condition) a similar cell proliferation occurs, the elements of which are very small in comparison with those from which they have developed, because under the changed stimulus they are kept embryonic and are compelled to divide soon after previous divisions, so that they can never reach maturity either in size

[1] Smith, Erwin F., Brown, Nellie A., and Townsend, C. O. Crown-gall of plants: its cause and remedy. U. S. Dept. Agr. Bur. Plant Indus. Bul. 213, 215 p., 36 pl. 1911.

Smith, Erwin F., Brown, Nellie A., and McCulloch, Lucia. The structure and development of crown-gall: a plant cancer. U. S. Dept. Agr. Bur. Plant Indus. Bul. 255, 60 p., 109 pl. 1912.

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or function as long as the stimulus lasts. These inoculations (on the Paris daisy) have brought out another interesting fact. As the tendency of young fundamental tissue (the growing point) is to form a stele in its center, so when the mature tissues of the stem cortex are brought under the new stimulus and begin to proliferate, in the manner of embryonic tissues, primitive but imperfect stele-forming tendencies are developed in the tumor. I have not seen an actual shoot produced by such a tumor; but sieve tubes and trachei are formed in it (out of descendants of cortex cells, be it remembered); and cross sections of some of these small tumors show that these stelar elements have a tendency to be arranged in the form of a closed structure (primitive stele), although often this is not pronounced. These superficial tumors have no connection with the xylem or phloem of the true stele, for in no case did the needle punctures enter as far as the phloem, much less the cambium, and serial sections show clearly that all of their structures (blastomous cells, trachei, and sieve tubes) have been developed wholly, out of cortex cells (probably cortex mother cells). After a few weeks such shallow tumors cease to grow, or develop very slowly, owing to imperfect nutrition (lack of all connection with the xylem and phloem of the plant).

Third, when the crowngall organism (hop strain) is inoculated into the leaf axils of young growing plants (species of Pelargonium, Nicotiana, Lycopersicum, Citrus, Ricinus, etc.) the buds of which are in a dormant state and which under ordinary conditions will continue dormant—namely, unless the top of the plant is removed—a new type of tumor develops, one hitherto not seen in crowngall. Inoculating in this way I have obtained tumors covered all over with diminutive, abortive leafy shoots, or flower shoots, if flower anlage have been disturbed. The shoots may be variously twisted, fused, and fasciated, as in the common house geranium (*Pelargonium* spp.) shown in Plate XVIII. This apparently is what happens: The growth of the tumor distorts the tissues, tearing the anlage into small fragments which are variously distributed and develop on or in the tumor into organs of a size proportional to the size of the included fragment—here as part of an ovary or anther, there as a shoot. These pathological shoots live but a short time and are quite unable to carry on the normal activities of the plant when the other leaves are removed. I have believed for a long time that fasciation must be due to a bacterial infection; but this is, I believe, the first time that anyone has obtained it by means of a pure-culture inoculation.

The results obtained by inoculating the upper leaf axils of young growing plants of the castor-oil plant (*Ricinus communis*) are prompt and quite as striking (Pl. XIX).

On tobacco plants (*Nicotiana tabacum*) these teratoid tumors, developed in leaf axils (Pls. XX and XXII), have also produced secondary tumors repeating the structure of the parent tumor. Such tumors have been obtained both in stems and leaves, especially when inoculations were

made early; and they contain, along with the proliferating tumor cells (blastomous cells), the same teratoid elements as the primary tumor. These are true daughter tumors, being connected back to the primary tumor by a tumor strand which is quite different both in structure and in location (Pl. XXI) from that occurring in the Paris daisy. The latter, it will be remembered, follows the line of the spiral vessels in the inner wood, and is parenchymatous in its structure, containing only here and there some vessels (scattered trachei). This tobacco tumor strand occurs in the cortex, consists almost entirely of vessels, and is a true stem (stele), although developed under a pathological stimulus, and in a part of the plant where no stele was ever seen before—namely, in the outer cortex, through which it can be traced (parallel to the long axis of the stem) for long distances and from which at intervals leafy tumors are sent to the surface of the plant. From its frequent proliferation in the form of tumors it is evident that parenchymatous (blastomous) elements must also occur in the strand, but they are not abundant. In fact, in the parts I have examined they are almost as infrequent as are trachei in the daisy strand. Cross sections and longitudinal sections of this remarkable tumor strand show it to have spiral vessels in its center, surrounded by trachei cut by ray cells, beyond which is a cylinder of cambium surrounded by a cylinder of phloem, containing well-developed sieve tubes. This tiny stele has no cortex or epidermis because it does not need any, being surrounded and sufficiently protected by the normal cortex of the tobacco stem. This is a phenomenon due apparently to my new manner of inoculation (into shoot anlage), because some years ago by inoculating internodally on tobacco stems I obtained and figured[1] tumors and a tumor strand in cortex corresponding to those found in the Paris daisy—that is, composed chiefly of small-celled parenchyma. The difference in results must therefore be due to difference in the kind of tissue inoculated, each developing pathologically according to its own growth tendencies.

Fourth, on some plants (which were tobaccos) I have also obtained leafy tumors by making my bacterial inoculations *in places where no bud anlage are known to exist*—for example, in the middle of leaves. Ordinarily when leaf tissue in tobacco grows, it only produces more leaf tissue;[2] but when the crowngall organism (hop strain) is pricked into midribs or side veins, tumors arise and a portion of them are leafy—that is, bear shoots. I have obtained 27 such leafy tumors on a single plant and several on a single leaf, all within a period of a few weeks (Pl. XXIII). It is easy to obtain them. The young leaves yield a larger proportion of such tumors than the older ones, and I have observed no shoot-bearing tumors on leaves which were fairly well developed when inoculated.

[1] Smith, Erwin F., Brown, Nellie A., and McCulloch, Lucia. The structure and development of crowngall: a plant cancer. U. S. Dept. Agr. Bur. Plant Indus. Bul. 255, pl. 102–103. 1912.

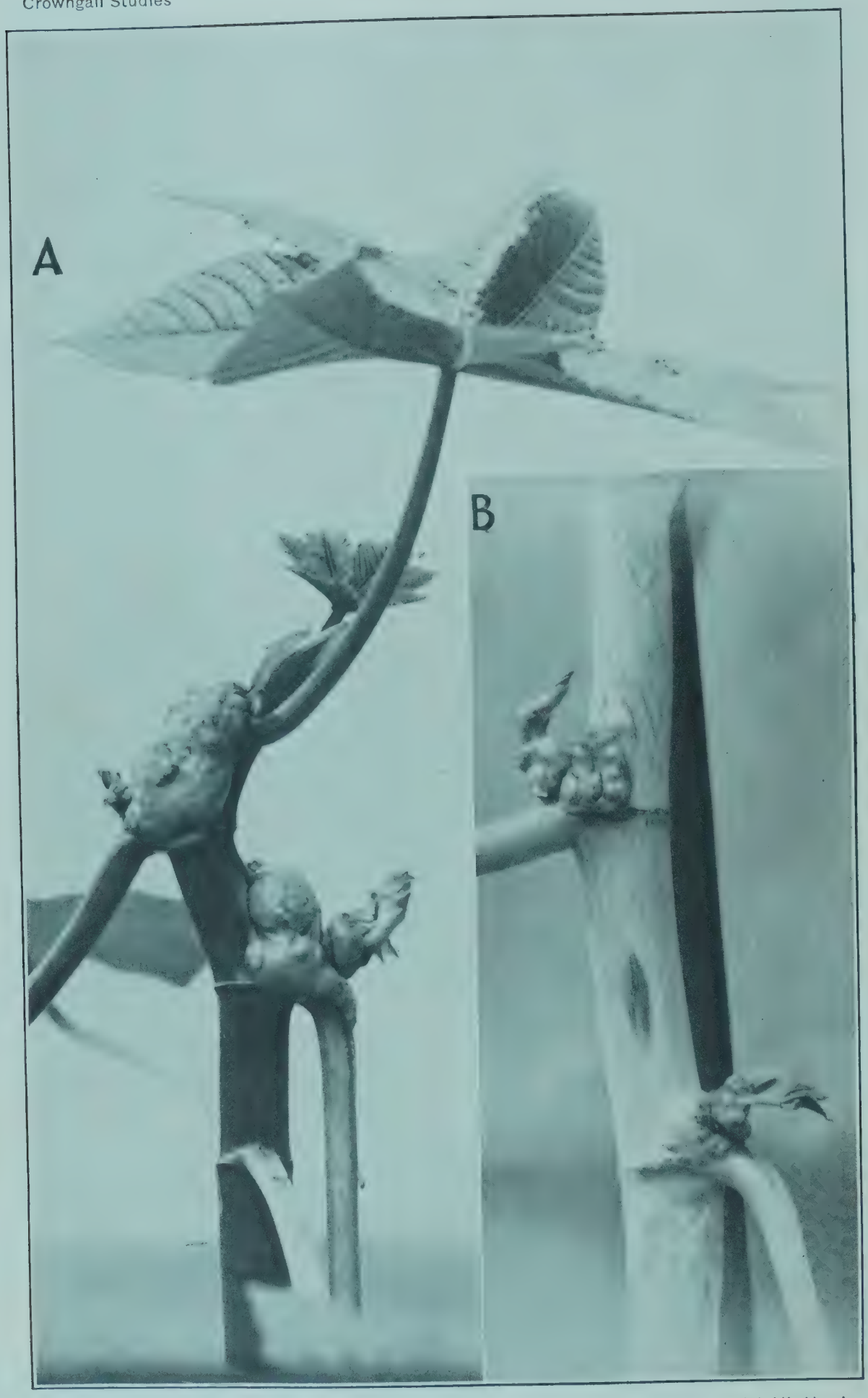
[2] I have never got any leaf cuttings of it to take root.

Rapidly developing young tissues seem to be necessary. Here again, a changed stimulus has produced a more embryonic and primitive condition, as shown by the appearance of these shoots. It is a pathological phenomenon, but one of more than passing interest, for, unless I am much mistaken, it has wide physiological and pathological bearings. It is another proof that the immature cell wherever it is located carries the inheritance of the whole organism, and that what it will finally become, as it matures, depends on the stimuli withheld from it or applied to it. In other words, it is so much evidence that any young cell may become a totipotent cell if it is subjected to the proper stimulus, and this stimulus may be either *physiological*, resulting in a normal structure, as when the top of a plant is removed and a new top grows in its place out of so-called adventitious buds (regeneration phenomena), or *pathological*, resulting in an embryonic teratoma, as when a tumor-producing schizomycete is introduced into sensitive growing tissues.

## PLATE XVIII

Teratoid crowngalls produced in *Pelargonium* spp. by inoculating *Bacterium tumefaciens* (hop organism through sunflower) into upper leaf axils on January 13, 1916. Photographed at the end of 74 days. At *X* the top of the shoot bearing five or six leaves was removed to show the tumor more distinctly. All of the leafy shoots here shown and many others too small to be seen distinctly in the photograph are outgrowths from the tumor, which also bears red abortive flower anlage. The upper shoot (*L*) was also flattened and fasciated (several shoots fused together) and the front leaves (*P P*) were turning yellow and dying.

L
P
P
X


A
B

## PLATE XIX

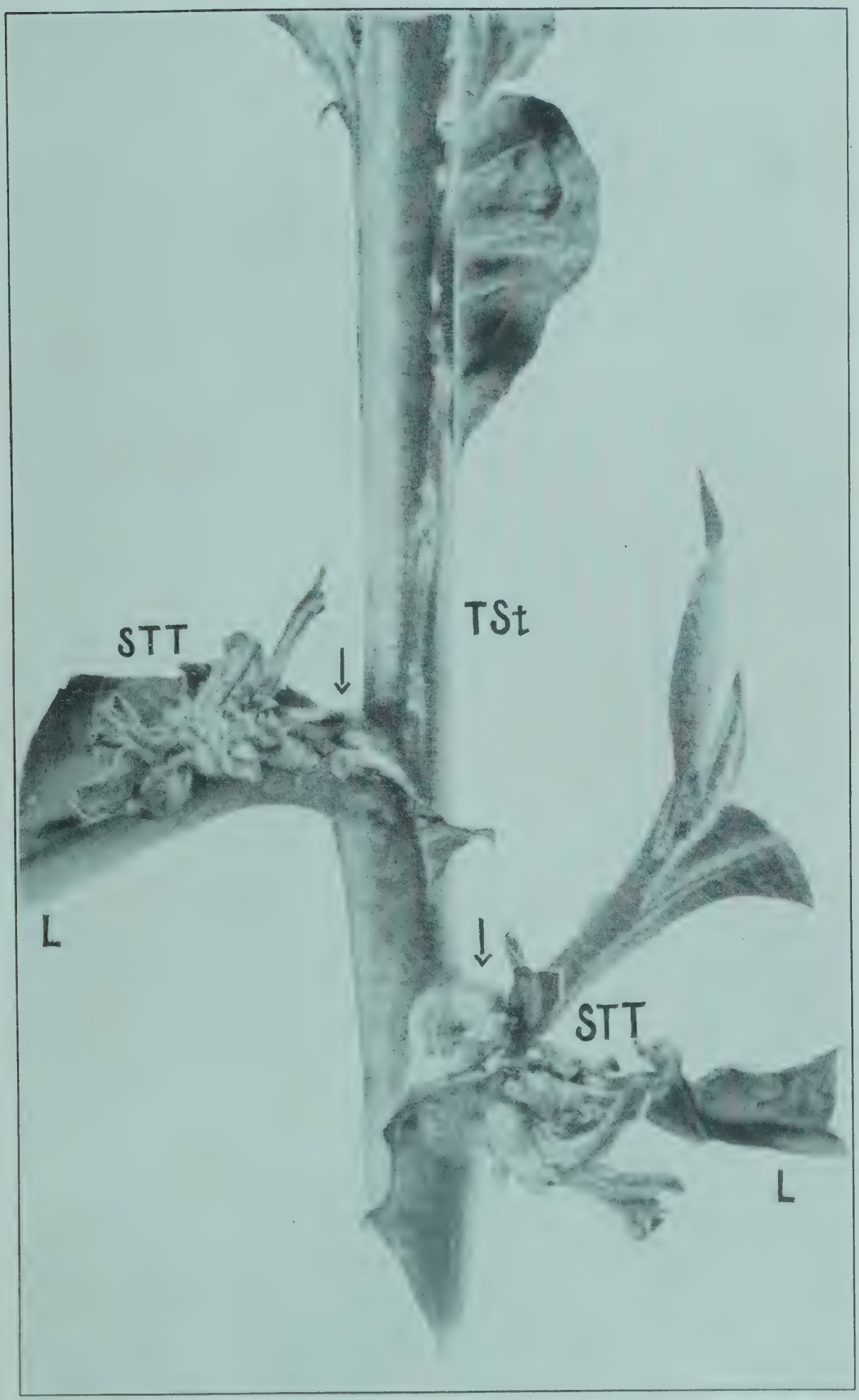
Teratoid crowngalls produced in castor-oil plant (*Ricinus communis*) by inoculating *Bacterium tumefaciens* (hop strain), the inoculations being made in the upper leaf axils of young, vigorous, unbranched plants.

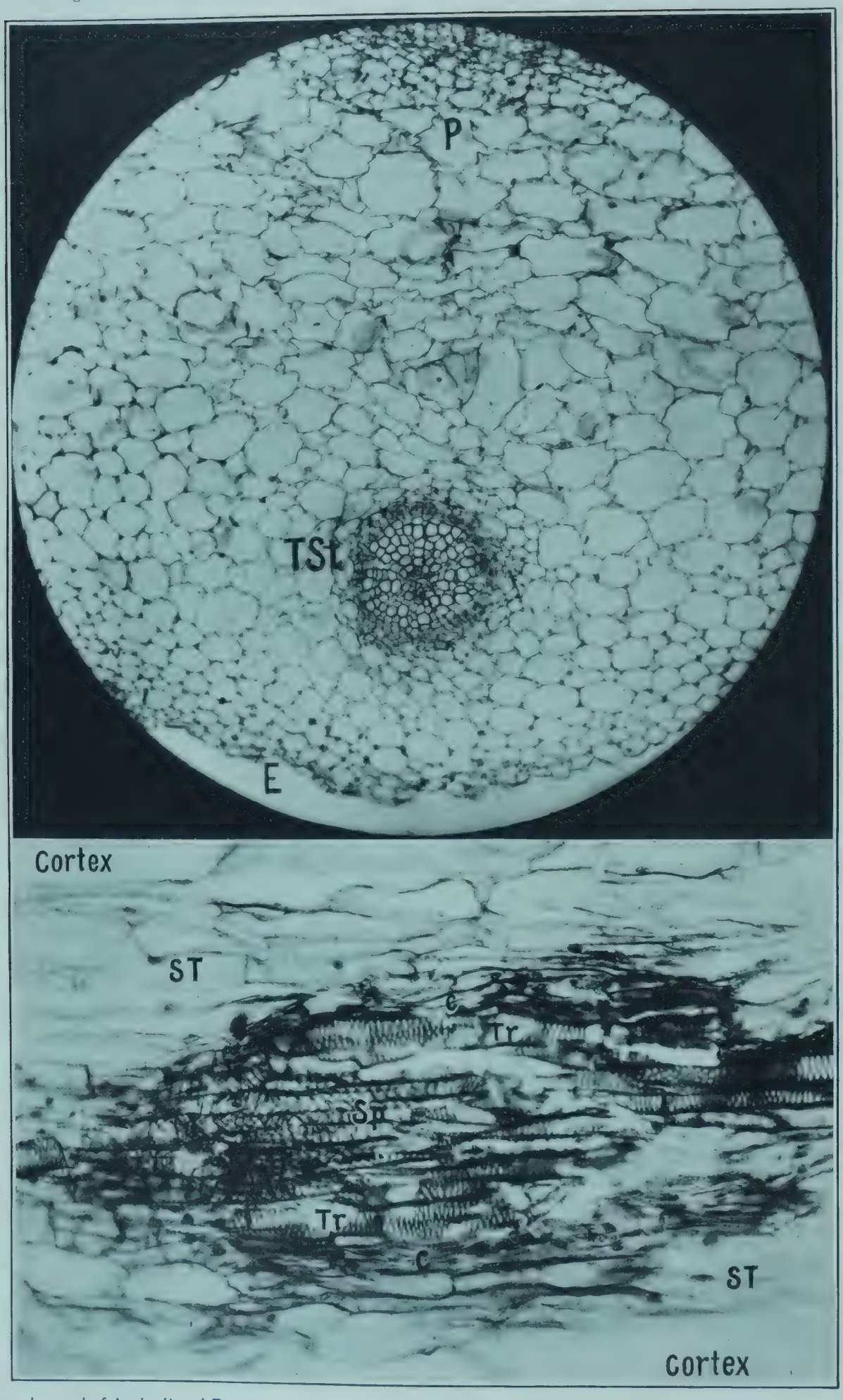
Fig. A.—A red-stem variety. Leaves reflexed; axis distorted; and feeble shoots developing out of the axillary tumors. There are on the tumors other smaller shoots not shown here distinctly. Time, 13 days.

Fig. B.—A green-stem glaucous variety. As in figure A, but time 17 days. Here also internal growths (root anlage) are pushing up the tissues of the stem below the lower leaf. A few days later these roots appeared on the surface, both of this internode and of the one above it. This phenomenon has been recorded previously by the writer as sometimes occurring on inoculated stems of the Paris daisy and other plants in the vicinity of developing tumors (Smith, E. F. Bacteria in Relation to Plant Diseases. vol. 2, fig. 26. 1911).

## PLATE XX

Teratoid crowngalls produced in tobacco by inoculating *Bacterium tumefaciens* (isolated from a hop tumor several years ago and passed through a sunflower in 1915). The inoculations were made by needle pricks in the axils of the lower leaves (under the arrows), at which places small leafy tumors developed. These sent tumor strands into the midribs of both leaves (*L L*) and later secondary teratoid tumors (*S T T*) burst through and covered the top of the midrib. From the upper leaf axil also a tumor strand developed, passing upward through 5 internodes and then out into the midrib of a leaf for several inches, giving rise at frequent intervals to tumors bearing leafy shoots (teratoid elements) and to others free from them. This tumor strand (*T St*) was not on the surface of the stem, as might appear from the photograph, but was near enough to show through as a translucent band about 2 mm. wide. Time, 26 days.


STT
TSt
L
STT
L


P
TSt
E
Cortex
ST
C
Tr
Sp
Tr
C
ST
cortex

## PLATE XXI

The teratoid tumor strand of Plate XX, which gives rise during its course to more than 30 small tumors.

Top. Cross section of outer part of right side of stem of tobacco plant shown on Plate XX. *P*, outer edge of the phloem; *E*, epidermis; *T St*, tumor strand, which is bedded in the normal cortex of the stem.

Bottom. Longitudinal section from upper part of the above tumor strand, more highly magnified, showing it to be a true stele. The coarse-celled tissue at top and bottom is the normal cortex of the stem. The pathological tissues are *S T*, sieve tubes; *C*, cambium; *Tr*, trachei; *Sp*, spiral vessels.

## PLATE XXII

Teratoid crowngalls produced in a tobacco plant by inoculating *Bacterium tumefaciens* (hop strain through sunflower) into the leaf axils. Small tumors soon appeared where inoculated and these are now covered with pale leafy shoots which have swollen (tumefied) bases and are beginning to die. The top was cut away on the 26th day, and the plant was unable to make a new one out of these pathological shoots, but has grown it (*X*) from an uninoculated lower leaf axil. Time, 73 days.

X
74

A
C
X
B

## PLATE XXIII

Teratoid crowngalls produced in tobacco leaves with the hop strain of *Bacterium tumefaciens* by local (leaf) inoculations—that is, inoculation in places where shoot anlage are not known to exist.

Fig. A.—Portion of an upper leaf showing four shoot-bearing tumors growing from upper surface of the inoculated midrib. Leaf inoculated February 16, 1916. Photographed on April 1.

Fig. B.—Same as *A*, but the leaf reversed and the midrib stripped of its blade to show two other shoot-bearing tumors which have developed from its under surface. Actual height of the tallest shoot, 1.5 cm.

Fig. C.—From middle of another leaf on the same plant as *A*, but further magnified and photo made on an orthochromatic plate to show the pale green character of the shoot as contrasted with the dark green of the surrounding leaf (which is also in shadow). This tumor and its shoot arise from a branch of the midrib, the latter in cross section being shown at *X*. A smaller teratoid tumor bearing two shoots (at either side of *C*) developed on the upper surface of the leaf and the one bearing the longer shoot on its lower surface. The actual length of this shoot was 1.5 cm. The leaf was curved downward and the shoot was growing out horizontally. Time, 45 days.

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# EFFECT OF CERTAIN SPECIES OF FUSARIUM ON THE COMPOSITION OF THE POTATO TUBER[1]

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## INTRODUCTION

Potato tubers (*Solanum tuberosum*) are subject to attack by various parasitic fungi. Some of these organisms invade the tuber, kill the cells, break down the cell walls, and cause, directly or indirectly, a more or less complete disorganization of the host tissue. What constituents of the potato are most easily destroyed by the fungus and what compounds can not be utilized by it either in respiration or to build up its own tissue are of considerable interest in the study of the physiology of parasitism. It was to obtain information on the effect of some potato tuber rot fungi upon the tissues of the host plant that the present study was planned and carried out. In this investigation the effect of *Fusarium oxysporum* Schlecht. and *F. radicicola* Wollenw. on the sucrose, reducing-sugar, starch, pentosan, galactan, and crude-fiber content of the potato was studied. Some experiments were duplicated also with *F. coeruleum* (Lib.) Sacc.

The three species of Fusarium just mentioned are all parasites on the potato tuber. Smith and Swingle (9)[2] considered *F. oxysporum* to be the cause of a serious rot of potato tubers. Wollenweber (10) did not agree with these writers, and contended that this fungus, while the cause of a wilt disease of the potato plant, was not able to rot the tubers. This conclusion of Wollenweber's has recently been disproved by Carpenter (4), who corroborates the findings of Smith and Swingle on this point. With this species and with *F. radicicola*, the latter considered by Wollenweber and by Carpenter to be the cause of a tuber-rot of considerable importance, the writer experienced no difficulty in obtaining well-rotted tubers in two to three weeks after inoculation.

[1] The work described in this paper was carried out in cooperation with the Office of Cotton and Truck-Crop Diseases. The writer thanks Mr. C. W. Carpenter, of that office, for cultures of the fungi used.

The writer's thanks are also due Mr. A. A. Riley, of the Office of Plant Physiological and Fermentation Investigations, for assistance in the experimental part of this study.

[2] Reference is made by number to "Literature cited," p. 196.

## EXPERIMENTAL METHODS

As the methods for sterilizing, sampling, and inoculating followed in this study were similar to those outlined in a study of the brownrot of the peach (7), they will be discussed here only briefly. The sterile tubers were sliced longitudinally into four parts with a flamed knife. Particular attention was given to obtaining portions of approximately the same weight and same proportionate amount of cortex and pulp. Each quarter was placed in a small wide-mouthed flask or large test tube which had been stoppered with cotton, sterilized, and weighed. The containers with the portions of potatoes were weighed again and the samples were ready for inoculation. Two of the quarters from each potato were inoculated from stock cultures of some one of the fungi used in these experiments and a small quantity of sterile water was added to each of the four containers. The four samples, two inoculated and the two corresponding control samples, were placed side by side at room temperature until the inoculated portions were well rotted. They were then prepared and analyzed. The difference between the sound and the rotted portions in the content of the compounds determined was considered to be due to the action of the fungus. All control portions infected at the time the samples were prepared for analysis and all inoculated portions infected with organisms other than those used in the inoculations were discarded.

All samples were prepared for analysis by cutting them into very thin slices with a sharp knife and washing them into the proper vessel. Precautions were observed, of course, that none of the juice or pulp should be lost. The methods of analysis for agricultural chemists[1] were usually followed in the determination of the various compounds. The sugars were extracted from the tissue with alcohol and determined as in the work on the brownrot of the peach. The method of extraction is the alcohol method of Bryan, Given, and Straughn (3), somewhat modified to suit the conditions of the experiment. The amount of cane sugar was in all cases calculated from the reducing power of the extract before and after inversion with acid.

The starch determinations in the preliminary experiments were made only by the direct acid-hydrolysis method using the finely ground potato which had been extracted with alcohol. In the work with the sound and the rotted portions of the tubers, series of analyses were also made by the diastase method with subsequent acid hydrolysis.[1] Tollen's phloroglucid method[1] was followed in all cases in the determination of the pentosans. The methyl pentosans were determined according to the method of Ellett and Tollens (6), by extracting the precipitated phloroglucid with alcohol. The galactans and the crude fiber were de-

[1] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr., Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

termined by the usual methods[1] in dry ether-extracted samples which had been ground. For the percentage of dry matter the sliced-up samples were placed in glass-stoppered weighing bottles and covered with alcohol. The alcohol was then driven off and the samples dried to constant weight. All data were calculated to the original wet weight of the potato used. The potatoes used in the experiment were smooth white potatoes usually purchased at the local market. The cultures of fungi used in the experiments were subcultures from Carpenter's cultures of *F. oxysporum* 3395 and 3315; *F. radicicola* 3113 and 3319, and *F. coeruleum* 3501.

## EXPERIMENTATION

To determine the amount of variation in content of the different compounds in the four quarters of the potato, series of preliminary analyses were carried out. In these the potato was sampled in the usual way, except that the portions were sliced immediately and prepared for analysis. The results of these analyses are shown in Tables I to VI.

TABLE I.—*Reducing sugar and sucrose content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

| Potato No. | Reducing sugar. | | | | Sucrose. | | | |
|---|---|---|---|---|---|---|---|---|
| | Quarter A. | Quarter B. | Quarter C. | Quarter D. | Quarter A. | Quarter B. | Quarter C. | Quarter D. |
| 43 | 0.10 | 0.11 | 0.09 | 0.11 | 0.04 | 0.02 | 0.04 | 0.02 |
| 44 | .06 | .06 | .07 | .06 | .04 | .04 | .03 | .03 |
| 46 | .17 | .14 | .14 | .19 | .02 | .03 | .04 | .04 |
| 49 | .02 | .02 | .03 | .02 | .03 | .03 | .03 | .04 |
| 87 | 0 | 0 | 0 | 0 | .07 | .07 | .07 | .06 |
| 88 | 0 | 0 | 0 | 0 | .06 | .05 | .06 | .05 |
| 105 | 0 | 0 | 0 | 0 | .05 | .08 | .07 | .06 |

TABLE II.—*Starch content of quarters of sound potatoes determined by the direct acid-hydrolysis method*

[Expressed as percentage of starch, wet weight]

| Potato No. | Quarter A. | Quarter B. | Quarter C. | Quarter D. |
|---|---|---|---|---|
| 71 | 17.88 | 19.52 | 17.07 | 17.26 |
| 70 | 16.43 | 15.35 | 15.64 | 16.04 |
| 47 | 15.04 | 16.04 | 16.00 | 14.50 |
| 50 | 18.56 | 17.16 | 16.54 | 17.27 |

TABLE III.—*Pentosan content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

| Potato No. | Quarter A. | Quarter B. | Quarter C. | Quarter D. |
|---|---|---|---|---|
| 19 | 0.51 | 0.43 | 0.46 | 0.48 |
| 28 | .37 | .35 | .35 | .37 |
| 47 | .41 | .40 | .37 | .40 |
| 207 | .51 | .48 | .48 | .51 |

[1] Wiley, H. W., ed. Op. cit.

TABLE IV.—*Galactan content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

| Potato No. | Quarter A. | Quarter B. | Quarter C. | Quarter D. |
|---|---|---|---|---|
| 118 | 0.025 | 0.030 | 0.034 | 0.027 |
| 135 | .028 | .020 | .027 | .029 |
| 120 | .034 | .024 | .030 | .025 |

TABLE V.—*Crude fiber content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

| Potato No. | Quarter A. | Quarter B. | Quarter C. | Quarter D. |
|---|---|---|---|---|
| 102 | 0.50 | 0.59 | 0.48 | 0.47 |
| 210 | .47 | .47 | .47 | .44 |
| 211 | .40 | .41 | .39 | .36 |

TABLE VI.—*Dry matter in the quarters of sound potatoes*

[Expressed as percentage of wet weight]

| Potato No. | Quarter A. | Quarter B. | Quarter C. | Quarter D. |
|---|---|---|---|---|
| 118 | 20.86 | 19.96 | 21.95 | 17.78 |
| 119 | 20.70 | 20.96 | 19.16 | 19.77 |
| 120 | 19.73 | 20.23 | 21.42 | 20.73 |
| 138 | 24.12 | 24.58 | 25.36 | 24.80 |
| 143 | 24.19 | 22.37 | 24.81 | 22.82 |

Tables I to VI show that there is considerable variation in the percentage of some of the compounds in different quarters of the same tuber, though usually the actual difference is not great. It is noticeable that two portions of the same tuber are more nearly alike in composition than samples from different potatoes. The method, therefore, which involves the comparison of the content of two quarters of the same potato is more accurate than one based on a comparison of the composition of two different potatoes. The experiments in which sound and rotted quarters were analyzed to determine the effect of the fungi upon the potato show that data from which definite conclusions may be drawn can be obtained by this method.

Inasmuch as the mycelium of the fungi was present in the rotted portions of the potatoes, it was of interest to determine what influence the compounds elaborated by these fungi would have on the apparent composition of the tuber. Quantities of mycelia of the two fungi *F. radicicola* and *F. oxysporum* were accordingly grown on potato extract. This medium was prepared by boiling sliced potatoes until they were soft, filtering the extract through cotton, and sterilizing it in suitable flasks.

The flasks of this medium were inoculated and the fungi allowed to grow for two or three weeks. The mat of mycelium was then removed, washed, dried, ground, and analyzed. The data obtained from these analyses, calculated as percentage of the dry weight, are given in Table VII.

TABLE VII.—*Amount of alcohol-insoluble substance reducing Fehling's solution when hydrolyzed with dilute hydrochloric acid, pentosans, methyl pentosans, galactans, and crude fiber in mycelium of Fusarium oxysporum and Fusarium radicicola*

[Expressed as percentage of dry weight]

| Species. | Alcohol-insoluble substance reducing Fehling's solution when hydrolyzed with dilute hydrochloric acid (as dextrose). | Pentosans. | Methyl pentosans. | Galactans. | Crude fiber. |
|---|---|---|---|---|---|
| *Fusarium oxysporum*.. | 34.58 | 2.53 | 0.73 | 0.86 | 21.8 |
| | 31.90 | 2.60 | .68 | .66 | 18.4 |
| *Fusarium radicicola*.... | 31.63 | 1.20 | 1.50 | .72 | 20.3 |
| | 31.48 | 1.20 | 1.50 | .64 | 17.6 |

It is apparent from Table VII that the fungi growing on the culture media prepared from potatoes produce pentosans, methyl pentosans, galactans, and a considerable quantity of substance which is insoluble in alcohol and reduces Fehling's solution when hydrolyzed with dilute hydrochloric acid. That this last-mentioned substance can not result from the hydrolysis of the pentosans is evident from the relatively small pentosan content of the mycelium. The amount of substance which is considered as crude fiber in the table is also quite marked. It is evident, then, that both fungi build compounds which may be expected to raise the content of pentosans, galactans, and other substances in the tissue of the potato when the fungi and host are analyzed together. It must be remembered, however, that the percentages given in Table VII are related to dry weight of washed fungus mycelium and that the content of mycelium in 25 gm. of wet weight of the potato rotted with either of these fungi would be small.

The general appearance of the rotted portion of potato was typical for tubers rotted with these fungi at laboratory temperatures (from 20° to 25° C.) in a saturated atmosphere—that is, it was a wetrot (4, p. 187). The skin apparently was uninjured and could have been removed entire in most cases. The inner portion was soft and generally disorganized. Microscopic examination showed that the cells of the interior were apparently free from each other, as if the middle lamellæ had been dissolved. The starch grains did not appear to have been eroded in the time allowed for the experiment. The method of preparing the quarters of potato for analysis has been described.

The starch and sugar determinations were usually made on the same portion by extracting the pulp with alcohol, the extract being used for

the sugar and the solid residue for the starch determinations. The effect of three species of Fusarium, *F. oxysporum*, *F. radicicola*, and *F. coeruleum*, on the starch and sugar content of sound and rotted quarters of the same tubers was studied. The data obtained from the determination of the sugars are shown in Table VIII.

TABLE VIII.—*Reducing sugar and sucrose content of the sound and rotted quarters of potatoes*

[Expressed as percentage of the original wet weight]

| Species of Fusarium and potato No. | Reducing sugar. | | Sucrose. | |
|---|---|---|---|---|
| | Rotted quarter. | Sound quarter. | Rotted quarter. | Sound quarter. |
| Infected with *Fusarium oxysporum:* | | | | |
| 160 | 0.04 | 0.31 | 0.10 | 0.66 |
| 159 | .04 | .28 | 0 | .67 |
| 158 | 0 | .44 | 0 | 1.03 |
| Infected with *Fusarium coeruleum:* | | | | |
| 149 | .13 | .40 | .12 | .39 |
| 150 | .04 | .47 | .24 | .50 |
| 151 | .17 | .37 | 0 | .66 |
| Infected with *Fusarium radicicola:* | | | | |
| 32 | 0 | .03 | .04 | .24 |
| 26 | 0 | .02 | .04 | .19 |
| 34 | 0 | .03 | .02 | .09 |
| 41 | 0 | .02 | 0 | .42 |

In Table VIII it may be seen that all three species of Fusarium used the sugars. In most cases practically all the sugar had disappeared from the rotted portion, the cane sugar being utilized almost if not quite as completely as the reducing sugars. That the fungi could use disaccharids directly—that is, without breaking them down to their constituent monosaccahrids—seemed unlikely. It was therefore probable that the fungi secreted enzyms which were capable of hydrolyzing cane sugar, and possibly maltose also. To determine this point, tests were made for sucrase and maltase in extracts of the mycelium of *F. oxysporum* and *F. radicicola*. The fungi were grown for about three weeks or until a thick mat of mycelium was formed on potato extract. The felt was then separated from the liquid, ground up in a mortar and digested for 48 hours under toluol. The extract was filtered off and portions of it added to solutions of the sugars of known concentration. Controls of the boiled extract were also prepared. After the preparations had been allowed to stand overnight at laboratory temperature the amount of reducing sugar was determined. It was found that in the preparations of unboiled extract the sugars, both sucrose and maltose, were inverted almost quantitatively. The boiled extracts were practically without effect. It is evident then that the two fungi secrete both sucrase and maltase.

The starch determinations in the sound and rotted portions of the same tuber were made by two methods, as has been said. The data obtained by the direct acid hydrolysis method are given in Table IX, while the results of the determinations by the diastase method with subsequent acid hydrolysis are shown in Table X.

TABLE IX.—*Starch content of sound and rotted quarters of potatoes infected with different species of Fusarium, as found by direct acid hydrolysis*

[Expressed as percentage of starch of the original wet weight]

| Potato No. | *Fusarium oxysporum.* | | Potato No. | *Fusarium coeruleum.* | | Potato No. | *Fusarium radicicola.* | |
|---|---|---|---|---|---|---|---|---|
| | Rotted quarter. | Sound quarter. | | Rotted quarter. | Sound quarter. | | Rotted quarter. | Sound quarter. |
| 165..... | 14. 40 | 13. 53 | 149..... | 19. 19 | 18. 69 | 34..... | 16. 18 | 15. 79 |
| 168..... | 14. 08 | 14. 67 | 150..... | 18. 72 | 17. 48 | 26..... | 15. 24 | 16. 60 |
| 180..... | 16. 16 | 14. 62 | 151..... | 22. 06 | 22. 22 | 32..... | 15. 15 | 16. 01 |
| | | | | | | 41..... | 16. 83 | 16. 85 |

TABLE X.—*Starch content of sound and rotted quarters of potatoes infected with different species of Fusarium as determined by the diastase method with subsequent acid hydrolysis*

[Expressed as percentage of starch, wet weight]

| Potato No. | *Fusarium oxysporum.* | | Potato No. | *Fusarium radicicola.* | |
|---|---|---|---|---|---|
| | Rotted quarter. | Sound quarter. | | Rotted quarter. | Sound quarter. |
| 25..... | 17. 77 | 16. 85 | 47..... | 17. 50 | 16. 66 |
| 33..... | 12. 50 | 11. 32 | 48..... | 16. 66 | 15. 16 |
| 27..... | 15. 32 | 14. 05 | | | |

The effect of the fungi upon the starch in the potatoes is in marked contrast to their action on the sugars. In Table IX, which gives the results of starch determinations by the direct acid-hydrolysis method, it may be seen that the starch content of the rotted portion appears to be higher in many cases than that of the corresponding sound quarter. In the determinations by the diastase method followed by acid hydrolysis the apparent starch content of the rotted portion is always higher, as shown in Table X. The fact that the fungi build up substances which are insoluble in alcohol and reduce Fehling's solution when hydrolyzed with dilute hydrochloric aicd, as shown in Table VIII, would account for any apparent increase in starch content in the rotted portion when the starch is determined by the direct acid-hydrolysis method. If the substances are also either soluble in hot water originally or made so by the diastase treatment, the apparent increase in starch content when the starch is determined by this method would be explained. In the diastase method the starch paste is liquefied by the action of the diastase,

then filtered, and the filtrate hydrolyzed with dilute hydrochloric acid. Some of the mycelium of these fungi was extracted with alcohol, and then dried and extracted with hot water. The extract was then filtered off, treated with hydrochloric acid exactly as in the acid hydrolysis of starch, neutralized and tested for reducing substances. A considerable quantity was found. The filtrate did not give a qualitative test for pentosans. The apparent increase in starch content in the rotted portions of the potatoes, then, is due to compounds laid down by the fungi. From the fact that only a small amount of mycelium of these fungi could be present in the rotted potato it would seem probable that if the starch were attacked to any extent the apparent starch content as obtained by acid hydrolysis would be lowered in all cases. To obtain further information on this point experiments were carried out to ascertain whether these fungi secreted diastase and if so, whether this enzym could break down the starch grains of the potato.

Extracts of the undried, ground mycelium of the two fungi, *F. oxysporum* and *F. radicicola*, were made with 50 per cent glycerin. These extracts were filtered after 24 hours through absorbent cotton and portions added to a 2 per cent solution of "soluble starch." Suitable controls were prepared and all preparations allowed to stand in an incubator under toluol at 30° C. for 48 hours. At the end of this time the starch was practically all broken down by the extracts of both fungi. Similar experiments were carried out with starch paste made from potato starch with positive results. The fungi then secrete diastatic enzyms. The experiments, however, did not prove that the diastases were able to attack the starch grains before they were broken down. Brown and Morris (2) have shown that malt diastase can not act on ungelatinized potato starch, though the starch grains of barley are readily eroded by it. Whether the enzyms in the extracts of the mycelium could erode the starch grains of the potato at room temperature was determined by placing some well-washed potato starch in extracts and allowing the preparations to stand under toluol. They were shaken up and examined from time to time, but no sign of erosion of the starch grains was evident even at the end of a week. The extracts used were tested on starch paste or "soluble starch" with positive results. Smith and Swingle (9) mention that the starch in the potatoes rotted with *F. oxysporum* was apparently not eroded. It is, of course, possible that the potato starch grains are very slowly attacked by the diastases of these fungi or that some inhibitor is present which prevents the action of the enzym on the starch in this condition at the temperature at which these studies were made. These points should be investigated. At present, however, the conclusion seems warranted in view of the evidence that the starch of the potato is not appreciably affected by the fungi.

From the fact that these fungi penetrate the cell walls or parts of the cell walls of the potato in living parasitically upon their host, their effect

on the constituents of the cell wall was considered of especial interest. The substances studied in this investigation which may be considered to be, in part at least, components of the cell walls are the pentosans, crude fiber, and galactans (5). Inasmuch as the fungi apparently do not affect the skin in rotting the potato, it was considered of interest to find out the relative distribution of the pentosans and crude fiber in the skin and inner portion of the potato. For these analyses the potatoes were peeled as thinly as convenient and determinations made on the weighed peeling and inner portion separately. The results of the pentosan determinations are given in Table XI.

TABLE XI.—*Pentosan content of the peeling and inner portion of potatoes*

[Expressed as percentage of pentosans, wet weight]

| Potato No. | Skin. | Inner portion. | Potato No. | Skin. | Inner portion. |
|---|---|---|---|---|---|
| 116 | 0.62 | 0.28 | 140 | 1.02 | 0.59 |
| 133 | .88 | .39 | 163 | .72 | .36 |
| 134 | .80 | .47 | 164 | 1.07 | .50 |

When the pentosan content is calculated as wet weight, it is about half as great in the inner portion of the tuber as in the skin. There is, nevertheless, a considerable amount of the furfurol-yielding compounds in the fleshy part of the potato. Inasmuch as the fungus has practically no effect on the skin, it is to be considered that practically all changes in the pentosan content that take place during rotting are in the inner portion of the tuber. The results obtained from the pentosan determinations on the sound and the rotted portions of the potato tubers are shown in Table XII.

TABLE XII.—*Pentosan and methyl-pentosan content of sound and rotted quarters of potatoes*

[Expressed as percentage of pentosans, wet weight]

| Potato No. | Sound quarter. | | | Rotted quarter. | | |
|---|---|---|---|---|---|---|
| | Total pentosans. | Pentosans. | Methyl pentosans. | Total pentosans. | Pentosans. | Methyl pentosans. |
| Infected with *F. oxysporum:* | | | | | | |
| 29 | 0.53 | 0.47 | 0.06 | 0.50 | 0.35 | 0.15 |
| 30 | .53 | .41 | .12 | .46 | .35 | .11 |
| 35 | .45 | .36 | .09 | .44 | .35 | .09 |
| 40 | .52 | .42 | .10 | .37 | .26 | .11 |
| Infected with *F. radicicola:* | | | | | | |
| 171 | .28 | .23 | .05 | .25 | .20 | .05 |
| 174 | .37 | .32 | .05 | .29 | .24 | .05 |
| 176 | .25 | .19 | .06 | .26 | .21 | .05 |

Table XII shows that the total pentosan content, which includes all furfurol-yielding matter, and the pentosan content, which is the total pentosan content after the methyl pentosans have been extracted, are higher in all but one instance in the sound portions of the tuber. There is slightly more variation in methyl pentosan content; it is the same or greater in the rotted as in the sound portion in all but two cases. The fungi evidently use the pentosans, but do not affect the methyl pentosans to any extent. It is to be remembered that these fungi build up both pentosans and methyl pentosans when growing on potato extract. The content of these substances, then, in the rotted portions given in Table XII is undoubtedly the difference between the amount of pentosans broken down by the fungi in the interior of the potato and the amount built up by the fungi. The destructive processes evidently proceed more rapidly than the constructive, and some of the pentosans of the potato are used either in respiration or in the building up of other compounds.

From the effect of the fungi on pentosans it was considered probable that enzyms which could hydrolyze these compounds were present in the mycelium. Experiments were undertaken to determine this point.

The experiments were carried out as described in a previous paper (8), except that the fungi were grown on potato extract instead of a synthetic medium with gum arabic as a source of carbon. Xylan from rye straw was used as a substrate. The results of these experiments are given in Table XIII.

TABLE XIII.—*Effect of boiled and unboiled extract of mycelium upon xylan from rye straw, as shown by alcohol-soluble furfurol-yielding material and substance reducing Fehling's solution. (0.2 gm. of xylan in each preparation was maintained at 30° C. for one week.)*

| Species of Fusarium. | Quantity of cuprous oxid derived from material reducing Fehling's solution. | | Quantity of alcohol-soluble furfurol-yielding substances as pentosans. | |
|---|---|---|---|---|
| | Unboiled. | Boiled. | Unboiled. | Boiled. |
| | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| *Fusarium radicicola* | 45.2 | 15.4 | 13.1 | 5.7 |
| | 44.8 | 14.8 | 13.1 | 5.7 |
| *Fusarium oxysporum* | 14.1 | 6.5 | 18.6 | 6.8 |
| | 16.4 | 6.5 | 14.6 | 6.8 |

It is evident from Table XIII that the extracts of the fungi are able to break down xylan prepared from rye straw to an alcohol-soluble compound which reduces Fehling's solution and which forms furfurol when boiled with hydrochloric acid. The fungi then secrete an enzym or enzyms which can break down xylan probably to xylose.

The crude fiber of the potato is undoubtedly a mixture of compounds, among which are some of the cell wall constituents, including whatever

cellulose may be present. The distribution of the crude fiber throughout the tuber is not as uniform as that of the pentosans, as is shown by a comparison of Tables XI and XIV.

TABLE XIV.—*Crude fiber content of the skin and inner part of the potato tuber*

[Expressed as percentage on both a wet weight and dry weight basis]

| Potato No. | Percentage of crude fiber, wet weight. | | Percentage of crude fiber, dry weight. | |
|---|---|---|---|---|
| | Skin. | Inner portion. | Skin. | Inner portion. |
| 210 | 1. 54 | 0. 25 | 11. 11 | 1. 16 |
| 211 | 1. 33 | .25 | 6. 61 | 1. 06 |
| 212 | 1. 20 | .36 | 7. 89 | 1. 82 |

From Table XIV it may be seen that the crude-fiber content of the peeling is 3½ to 6 times greater than that of the inner portion calculated on a wet weight basis and from 4 to 10 times greater on the basis of dry weight. The inner portion of the potato contains usually a lower percentage of crude fiber than of pentosans.

The determinations of crude fiber on the sound and rotted portions of the potato tubers are given in Table XV.

TABLE XV.—*Crude-fiber content in sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

| Rotted with *Fusarium radicicola.* | | | Rotted with *Fusarium oxysporum.* | | |
|---|---|---|---|---|---|
| Potato No. | Rotted quarter. | Sound quarter. | Potato No. | Rotted quarter. | Sound quarter. |
| 37 | 0. 56 | 0. 54 | 177 | 0. 71 | 0. 58 |
| 39 | .57 | .56 | 178 | .73 | .62 |
| 115 | .40 | .37 | 179 | .69 | .62 |

The crude-fiber content is always higher in the rotted quarter of the tuber than in the corresponding sound portion, though the difference is not great. As has been mentioned earlier in this paper, the fungus builds up a considerable quantity of substance which is not dissolved in either the acid or alkali used in the crude-fiber determination; to this is due the rise in the crude-fiber content of the potato during rotting. It is possible, of course, that the fungi may break down the crude fiber of the host plant and build up some similar substance with greater rapidity. From the evidence brought out in these experiments, then, it is impossible to draw definite conclusions.

The substances in the potato which give mucic acid when boiled with proper concentration of nitric acid are considered in this study as galactans. They are present in small quantities in the potato, and the com-

bination in which they occur in the tuber was not investigated. Galactose might occur in combination with raffinose, in a glucoside or combined in the cell walls. It probably occurs in plants most commonly in the last-mentioned combination. The effect of the fungi upon the galactan content of the potato is shown in Table XVI.

TABLE XVI.—*Galactan content of sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

| Rotted with *Fusarium radicicola.* | | | Rotted with *Fusarium oxysporum.* | | |
|---|---|---|---|---|---|
| Potato No. | Rotted quarter. | Sound quarter. | Potato No. | Rotted quarter. | Sound quarter. |
| 27 | 0.039 | 0.062 | 166 | 0.069 | 0.071 |
| 31 | .033 | .060 | 167 | .068 | .076 |
| 42 | .029 | .030 | 172 | .081 | .083 |

It is evident from the table that the fungi lower the galactan content of the potato. The fungi produce galactans when growing upon potato extract and the data in Table XVI show that the breaking down process proceeded faster than the building up.

The amount of dry matter of the sound and rotted quarters determined as mentioned earlier in this paper is shown in Table XVII.

TABLE XVII.—*Amount of dry matter in sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

| Rotted with *Fusarium radicicola.* | | | Rotted with *Fusarium oxysporum.* | | |
|---|---|---|---|---|---|
| Potato No. | Rotted quarter. | Sound quarter. | Potato No. | Rotted quarter. | Sound quarter. |
| 27 | 20.83 | 21.19 | 166 | 17.73 | 18.91 |
| 31 | 19.88 | 22.59 | 167 | 18.93 | 20.45 |
| 42 | 20.98 | 22.13 | 172 | 18.17 | 19.36 |

As was to be expected, the rotting of the potato by the fungi lowered the percentage of dry weight as calculated to the original weight of the portion of the potato used in the experiment. This is probably due to an increased respiration—that is, a respiration of the quarter of the potato plus the respiration of the fungus which in a given time is greater than a portion of the same potato alone.

## DISCUSSION

From the foregoing pages it is evident that the tuber-rot fungi used in this study considerably alter the composition of the potato. That they should be able to utilize the sugars of the potato was to be expected. Most fungi use glucose readily as a source of carbon. Behrens (1) has shown that *Sclerotinia fructigenia* lowers the sugar content of apples in

rotting them. The brownrot fungus of peaches reduces the sugar content of that fruit. The presence of the enzyms sucrase and maltase in fungi has frequently been recorded.

The starch content of the potato makes up the greater part of its dry weight and may be regarded as stored food material. That the fungi which so efficiently utilize the monosaccharids and disaccharids of the potato tuber are unable, apparently, to affect this polysaccahrid is of considerable interest. The fungi grow for the most part in the cell walls and thus are not closely in contact with the starch grains. This might retard the action because of the low rate of diffusion of the diastase but could hardly inhibit it entirely. The fact that the diastases of these fungi had no apparent effect on unbroken starch grains *in vitro* during the time of the experiment, while potato starch when gelatinized was readily hydrolyzed by these enzyms, indicates that the rate of action under what are usually favorable conditions for such reaction is to say the least very low. The experiments seem to show that enzymic studies are of doubtful value in determining the effect of the parasite on the host plant unless corroborated in a study of the physiological relations existing between the two organisms. The effect of the fungi on the pentosan and galactan content of the potato shows that they can break down at least some of the constituents of the cell wall. Now, when a parasitic fungus such as those used in this study enters a cell of its host plant, it must either force its way in mechanically by exerting sufficient pressure to rupture the cell wall or a portion of the cell wall must be dissolved. Likewise, in growing between the cells of the host plant where no appreciable intercellular spaces exist, the cells must be forced apart mechanically or some parts of the cell walls dissolved. It is evident from their effect on the pentosans that these fungi are able to dissolve at least some portions of the cell wall. That they secrete enzyms which can hydrolyze xylan is more evidence on this point. The crude-fiber content of the potato was increased in rotting owing to the formation in the fungi of some substances which were not broken down by the acid or alkali treatment in the crude-fiber determinations. Therefore it was impossible to obtain evidence as to the effect of the fungi upon the crude fiber. As shown in the tables the crude-fiber content of the inner portion of the potato is not high. It is noticeable that throughout this study the different species of Fusarium had practically the same effect on the potato.

## CONCLUSION

In conclusion, it has been shown in this study that the fungi in the potato reduced the content of sugar, both sucrose and reducing sugar, pentosans, galactans, and dry matter. The starch and methyl pentosans are apparently not affected appreciably, and the crude-fiber content was not reduced. It was shown that these two species of fungi secrete sucrase, maltase, xylanase, and diastase; the last-mentioned enzym is apparently incapable of acting on the ungelatinized potato starch.

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# HYPERASPIS BINOTATA, A PREDATORY ENEMY OF THE TERRAPIN SCALE

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## INTRODUCTION

One of the most effective enemies of lecanium scales is the coccinellid beetle *Hyperaspis binotata* Say. Its economic importance was impressed on the writer during the seasons of 1912 and 1913, when he was studying the life history and control of the terrapin scale (*Eulecanium nigrofasciatum* Pergande). Throughout the spring and early summer the larvæ, conspicuous by their flocculent covering, could be found in large numbers feeding upon the immature scales and overturning the adult scales. The adult beetles do not feed upon the mature scales, but they destroy the young and also attack aphides, or plant lice, and other soft-bodied insects. In view of the economic importance of this beetle a study of its life history was undertaken at the suggestion of Dr. A. L. Quaintance, in charge of Deciduous Fruit Insect Investigations, Bureau of Entomology. The work was begun in the summer of 1912 and completed in 1913.

## HISTORICAL SUMMARY

Very little has been written about *Hyperaspis binotata*. Say (1, p. 303),[1] in 1826, described the male under the present name, and the female as *Coccinella normata*. G. R. Crotch (2, p. 380) considered the form with the subapical red spot as a variety of *H. signata* Olivier, and gave as synonyms *H. binotata* Say, *H. normata* Say, and *H. leucopsis* Melsheimer.

T. L. Casey (3, p. 124), in 1899, considered *H. binotata* Say as a distinct species and gave the following synonymy: *H. signata* Le Conte, *H. normata* Say, *H. affinis* Randall, and *H. leucopsis* Melsheimer.

J. G. Sanders (4, p. 3), in 1905, mentions *H. binotata* as a valuable predatory enemy of *Pulvinaria* spp. J. B. Smith (5, p. 606; 6, p. 570), in the same year, reported the same species as reducing an infestation of *Pulvinaria* spp. at Montclair, N. J., from 500 to 1,000 scales to a leaf to about one dozen scales to a leaf.

S. A. Forbes (7), in his annual report for 1908, mentions the species as one of the principal enemies of *Pulvinaria* spp. in Illinois. In 1910, W. S. Blatchley (8, p. 523), gives a key to the species of Hyperaspis found in Indiana and remarks that *H. binotata* Say is "a variety of *H. signata* Oliv., having the subapical spot lacking, color and structure otherwise exactly as in that species." W. E. Britton (9, 8), in 1914, treats this species,

[1] Reference is made by number to "Literature cited," p. 203.

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mentioning it as a great destroyer of the cottony maple scale (*Pulvinaria vitis* Linnaeus) and stating that it feeds upon both the woolly maple-leaf scale (*Phenacoccus acericola* King) and the tulip scale (*Eulecanium tulipiferæ* Cook).

These references bring the history of the species down to the date of the present paper, which deals with the life history and habits of the species when feeding upon the terrapin scale.

## DISTRIBUTION

*H. binotata* occurs in most of the territory east of the Mississippi River and extends west of this river in some States to the semi-arid region. It is most abundant in the Atlantic States from Connecticut to Maryland, but is common from New Jersey to Illinois. All localities known to the writer are indicated upon the map (fig. 1).

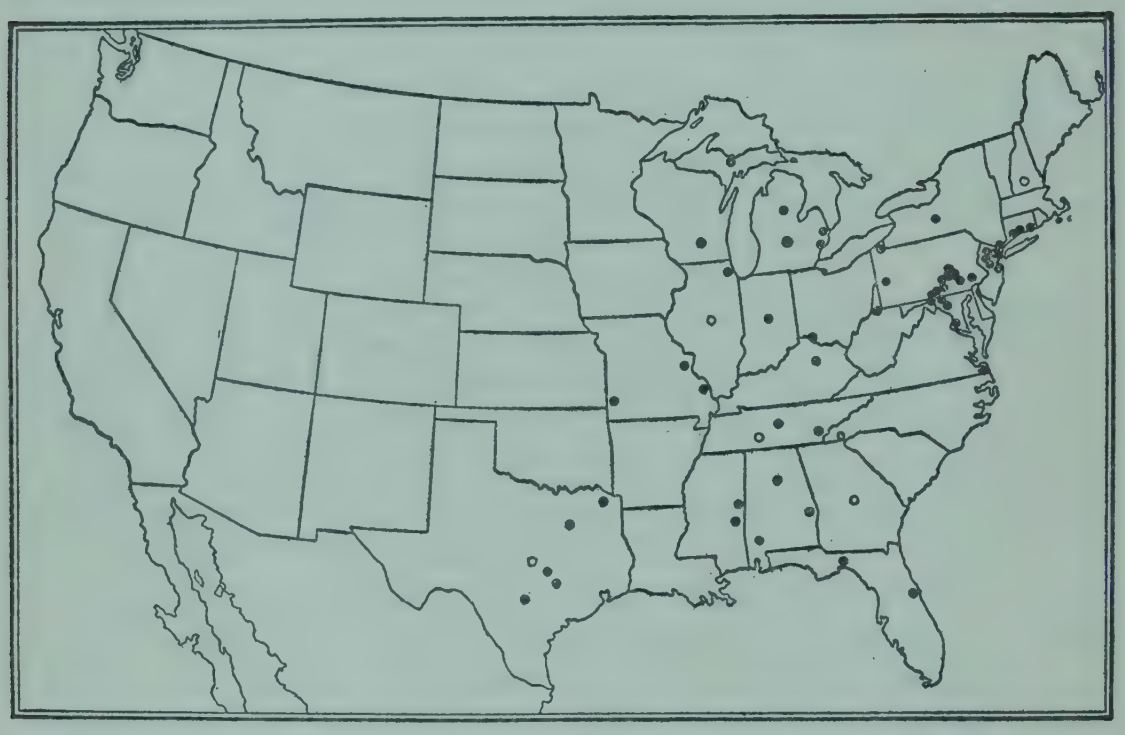

FIG. 1.—Map showing the distribution in the United States of *Hyperaspis binotata*: ●=definite record; ○=doubtful record.

## HOSTS

*H. binotata* feeds upon honeydew, aphides, aphis eggs, and mealy bugs and other soft-bodied scales. The larvæ, so far as observed, feed upon scale larvæ and young scales. They seem to have preyed originally upon species of Pulvinaria, to the egg masses of which the larvæ have a superficial resemblance. The species thrives upon the terrapin scale and seems to be rather more abundant where it preys exclusively upon this scale.

## DESCRIPTION OF LIFE STAGES

### IMAGINAL STAGE

The adult (Pl. XXIV, fig. 1, 2) is a small hemispherical beetle which passes the winter in rubbish or under bark. It was described by Say (1) in 1826 from the male as follows:

"Black, lateral margin of the thorax and head yellow; each elytron with a rufous spot; body rounded-oval, convex, punctured, black, polished; head pale yellow, labrum and transverse line on the vertex piceous; thorax with a yellow margin extending for a short distance on the anterior margin; anterior margin with an obsolete yellowish line interrupted in the middle; elytron each with a rufous, orbicular, central spot."

### EGG STAGE

The egg (Pl. XXIV, fig. 3), which was first obtained by the writer in 1913, is oblong-elliptical and somewhat depressed; 10 specimens measured from 0.6 to 0.775 mm. in length (average, 0.704 mm.) and from 0.218 to 0.4 mm. in width (average 0.312 mm.). In color it is light salmon, changing ultimately to ash-gray; the shell is membranous, becoming indented with age. Hatching takes place through a longitudinal slit on the upper surface.

### LARVAL STAGE[1]

The first instar has characteristic markings, and represents a rather primitive type of coccinellid larva. The other instars are similar to the first, but they are covered by a white fleece of wax filaments which masks their characters.

First instar (Pl. XXIV, fig. 4).—Length 1.22 mm. (1.125 to 1.275 mm.), width 0.478 mm. (0.450 to 0.575 mm.); body grayish white, semiopaque, cylindrical, and tapering caudad. Head black, with a white trident spot over the epicranial and frontal sutures; three pairs of ocelli present; length 0.125 mm., width 0.225 mm. Thorax sparsely pilose, the segments each with a pair of black dots; prothorax with two black clouded areas surrounding, but mainly cephalad of the dots. Abdominal segments each with a row of eight hairs and a pair of long lateral setæ; ninth segment black above; tenth segment, the so-called anal lobe, retractile.

Second instar (Pl. XXIV, fig. 3, *a*).—Length 2.5 mm. (1.3 to 2.75 mm.), width 1.08 mm.; body yellowish white, pubescent and covered with a white fleece. Head black with the trident spot mildly obscured; length 0.175 mm., width 0.325 mm. Thorax white, immaculate; legs gray, marked with black. Abdomen devoid of conspicuous lateral setæ.

Third instar.—Length 2 to 3.38 mm., mostly 2.5 mm.; width 0.9 to 1.75 mm., mostly 1.125 mm. Head black, pigmentation on the posterior part of labium confluent; length 0.275 to 0.3 mm., width 0.45 to 0.5 mm., mostly 0.475 mm. Abdomen with eight pairs of conspicuous blood pores. Otherwise as in the second instar.

Fourth instar (Pl. XXV, fig. 1, 2).—Length 2.5 to 6.25 mm., mostly 5.5 mm.; width 1.125 to 2.5 mm., mostly 2.25 mm. Body subglobose, yellowish gray. Head glabrous, white, flecked with black, pigmentation on the posterior part of labium not confluent on the median line; length 0.3 to 0.375 mm., mostly 0.35 mm.; width 0.575 to 0.65 mm., mostly 0.6 mm. Otherwise as in the third instar.

### PUPAL STAGE

Pupa (Pl. XXV, fig. 3, 4) inclosed within the larval skin; length 2.03 to 4.19 mm., mostly 3.9 mm.; width 1.77 to 1.86 mm.; color uniform chestnut-brown; ovate, with a depressed segmented area on the dorsum; dorsal surface hispid; ventral surface mildly pilose.

[1] A detailed morphological study of this larva by Dr. Adam Böving is in course of preparation.

## HABITS AND SEASONAL HISTORY

### THE BEETLES

The beetles emerge from hibernation at Mont Alto, Pa., about the middle of April and commence mating about the 20th of that month. When the species is feeding upon the terrapin scale, the beetles hibernate for the most part at the bases of scale-infested peach (*Amygdalus persica*) trees. After emerging from hibernation they soon depart in search of food and do not return to the peach until the adult scale, which the beetle is unable to destroy, begins to deposit honeydew—about the middle of May. For the rest of the season the species remains upon the peach, feeding upon the scale and its honeydew. The overwintering beetles are nearly all dead by the middle of July, while the new brood of beetles escapes from pupæ for the most part during the first half of that month.

There is some indication of a second brood, but there is not enough evidence at hand to establish it.

### THE EGGS

A very typical group of four eggs just as they were deposited is shown in Plate XXIV, figure 3. It will be noticed that the eggs are not clustered, but are placed more or less at random in the irregularities of the bark adjacent to the host. The terrapin scale upon which the species was feeding is found only upon young wood, the growth rings of which supply a convenient shelter for the eggs of the beetle. It is not unusual, however, to find eggs in crevices at the base of fruit spurs or even upon smooth bark. It is worthy of note in this connection that the eggs are not placed under the scales. It was found that the membranous shell became dry and shriveled in from three to six days, and that the egg changed to an ash-gray near the end of the incubation period.

The first eggs of the season were laid upon the twigs of scale-infested peach trees at Mont Alto, Pa., on May 3, 1913, but were immediately consumed by the beetles, as were all later eggs, until the food supply became abundant. It was not until May 26 that eggs were permitted to hatch. Oviposition reached its maximum about June 5, and continued in a small way until September 1. Owing to the tendency of the beetles to devour their eggs, it was not possible to determine definitely the beginning of oviposition or the total number of eggs; 36 was the largest number obtained from a single female, but there were indications that several times that number had been deposited. Incubation lasts from six to eight days; the average for 18 eggs deposited between June 27 and 30, 1913, was seven days.

THE LARVÆ

The larvæ at the time they escape from the egg have the pigment lacking from the head, legs, and ninth abdominal segment. They begin searching at once for the terrapin scales; and when one is found, a larva enters the brood chamber through the anal cleft, where it remains during the first and second instars. The first noticeable appearance of the coccinellid larvæ in the orchard, which occurs about June 18, coincides with the beginning of reproduction of the terrapin scale. Once within the brood chamber of a scale the coccinellid larva (Pl. XXIV, fig. 4) preys upon the new-born young of that particular scale until the end of the second instar, by which time the rapidly growing coccinellid displaces the scale.

The second molt is made in the open, mostly at the base of a fruit spur. In the third and fourth instars many mature scales are destroyed, being displaced (Pl. XXIV, fig. 5) by the coccinellid larvæ as these thrust their heads into the brood chambers to secure the young scales. When all the old scales have been destroyed, the ladybird larvæ, which now have a superficial resemblance to mealy bugs, migrate to the leaves and there continue to feed upon such of the scale larvæ as were able to reach the leaves. It is estimated that a single coccinellid larva will destroy 90 mature scales and 3,000 larvæ.

The length of the larval instars, together with the number of specimens used in their determination, is shown in Table I.

TABLE I.—*Length of the larval instars of Hyperaspis binotata*

| Instar. | Number of specimens. | Length of instar. | | |
|---|---|---|---|---|
| | | Average. | Minimum. | Maximum. |
| | | *Days.* | *Days.* | *Days.* |
| First | 17 | 2.98 | 2 | 4 |
| Second | 11 | 2.18 | 1 | 3 |
| Third | 7 | 2.71 | 2 | 4 |
| Fourth | 5 | 12.00 | 12 | 12 |

The dates at which the respective instars occur in the field are given in Table II. The first and second dates show the time of greatest abundance; the first and last dates show the total time of occurrence for each instar.

TABLE II.—*Sequence of the seasonal appearance of the larval instars of Hyperaspis binotata in the field*

| Instar. | Date present in field. |
|---|---|
| First | June 17 to 20 to Sept. 15. |
| Second | June 20 to 22 to Sept. 20. |
| Third | June 22 to 25 to Sept. 25. |
| Fourth | June 25 to July 7 to Sept. 30. |

The author has depended upon head measurements in distinguishing the instars; a key for this purpose (Table III) has proved satisfactory. As will be seen from the table, it is only necessary to consider the width of the head.

TABLE III.—*Key for determining the larval instars of Hyperaspis binotata according to width of head*

| Instar. | Width of head. |
|---|---|
| | *Mm.* |
| First..... | 0.225 |
| Second..... | .325 |
| Third..... | .475 |
| Fourth..... | .600 |

## THE PUPA

The pupal period lasts for from 10 to 13 days, averaging 12 days. Pupæ appear in the field early in July and are most abundant from the 7th to the 20th of the month. They are found, surrounded by the last larval skin, attached to leaves or concealed in clusters under bark. An occasional one may be found as late as October.

## NATURAL ENEMIES

There seem to be very few enemies of this ladybird. No parasites were obtained, and no birds were observed to feed upon it. Aphis lions were found preying upon the eggs, and a common plant bug, *Brochymena* sp., was taken upon two occasions with this coccinellid impaled upon its beak.

## SUMMARY

*Hyperaspis binotata* Say is found in the eastern United States and westward to the semiarid region. It feeds upon aphides and soft-bodied scales and is very effective in controlling the cottony maple scale and the terrapin scale. The eggs are salmon-colored and are deposited singly on twigs adjacent to the hosts. The life cycle requires 39 days and is as follows: Incubation, 7 days; first instar, 3 days; second instar, 2 days; third instar, 3 days; fourth instar, 12 days; pupa, 12 days.

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## PLATE XXIV

*Hyperaspis binotata:*

Fig. 1.—Male, showing the characteristic markings. Much enlarged.

Fig. 2.—Female, showing the dorsal view. Much enlarged.

Fig. 3.—Eggs and a second-instar larva. *a*, Second-instar larva as disclosed by displacing the host; *b*, larvæ of the terrapin scale, *Eulecanium nigrofasciatum; c*, a displaced scale; *d*, eggs "in situ"; *e*, egg somewhat enlarged.

Fig. 4.—First-instar larva.

Fig. 5.—Method of attacking the mature scales during the third and fourth instars.

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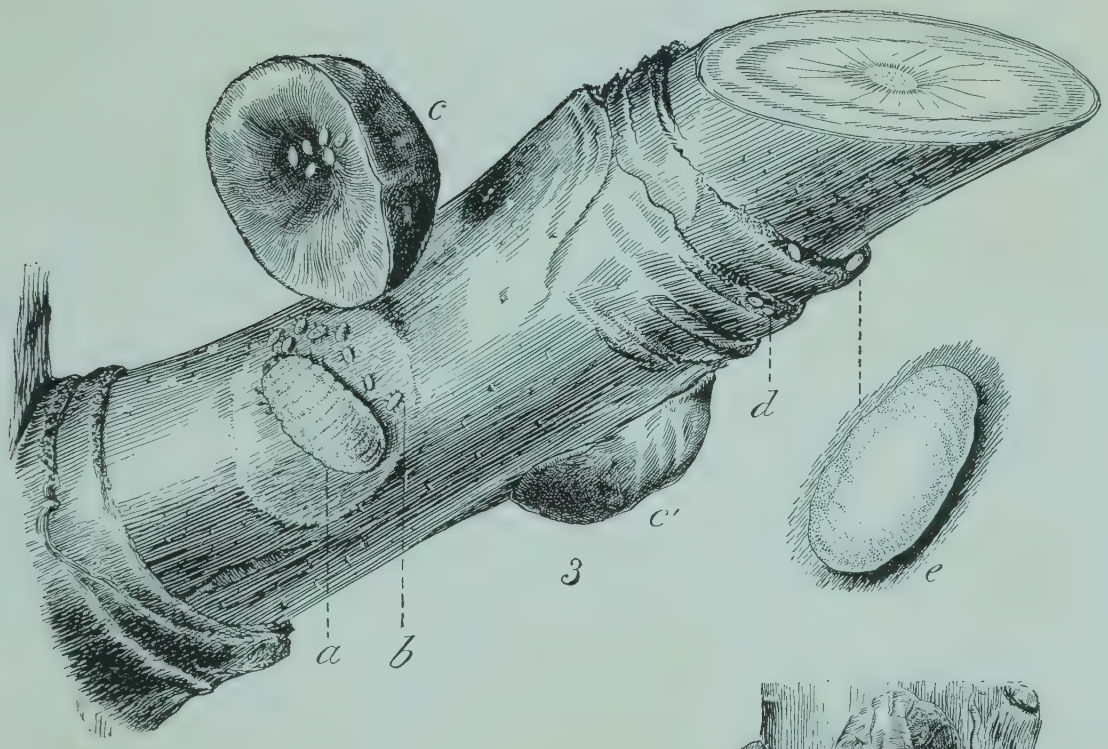
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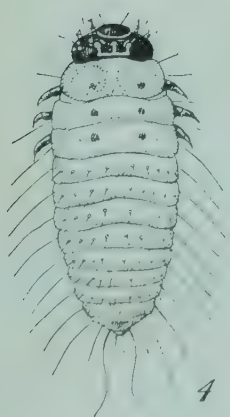
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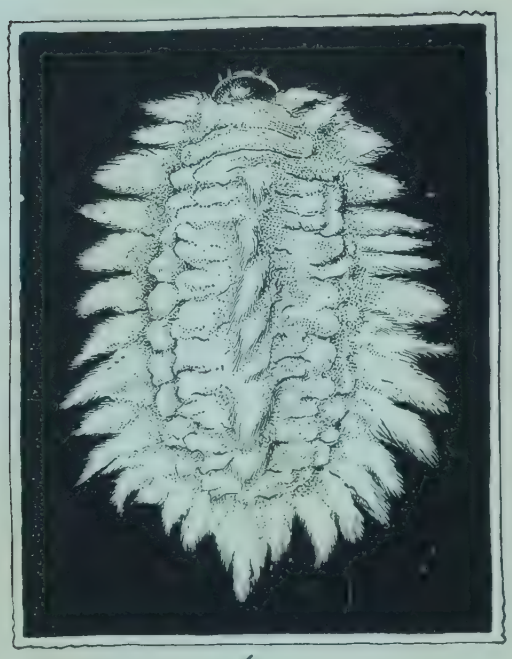
Hyperaspis binotata

PLATE XXV



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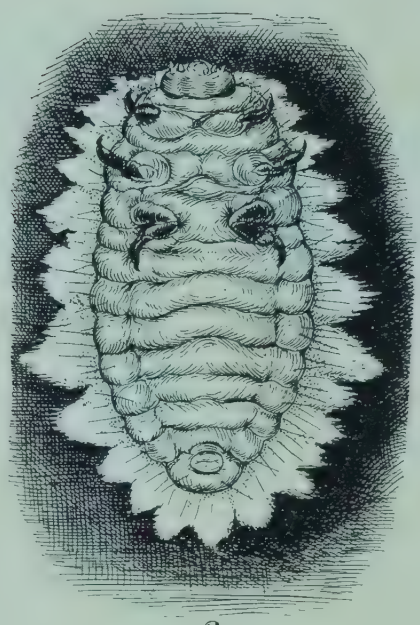

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## PLATE XXV

*Hyperaspis binotata:*

Fig. 1.—Mature larva as it appears when attacking the leaf-attached larvæ of the terrapin scale, *Eulecanium nigrofasciatum.*

Fig. 2.—Ventral view of mature larva.

Fig. 3.—Dorsal view of pupa, showing the last larval molt skin and the depressed segmented area.

Fig. 4.—Ventral view of pupa.

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# TESTS OF THREE LARGE-SIZED REINFORCED-CONCRETE SLABS UNDER CONCENTRATED LOADING

By A. T. GOLDBECK, *Engineer of Tests*, and E. B. SMITH, *Associate Mechanical Engineer, Office of Public Roads and Rural Engineering*

## INTRODUCTION

Numerous instances occur in reinforced-concrete design in which the use of slabs supported at two ends only is required, and in many such cases the critical loading is concentrated at one or more points. Such a condition may exist on slab-bridge floors, box culverts, on floors of buildings where heavy machinery is housed, and in other constructions where loads are concentrated.

If a slab, supported at two ends and carrying a single concentrated load, is imagined to be divided into narrow strips extending from support to support, it would seem reasonable to assume that the strip immediately under the load carries a very large part of it and that the adjacent strips receive a smaller amount, depending upon their distances from the load. The most remote strips, those at the edges of the slab, would then probably receive very little load. The question which concerns the designer of such a slab is that of the relative magnitude of the stresses at different distances from the load.

Up to a few years ago the technical literature on this subject was practically nonexistent, and the result was that engineers relied largely on their judgment when called upon to design slabs subjected to concentrated loads. Very naturally, large variations in load-distribution assumptions were made, and as a consequence there were great differences in the design even when the span and load to be carried were practically identical.

The necessity for definite knowledge on this subject was very forcibly brought to the attention of the engineers of the Office of Public Roads and Rural Engineering a few years ago, and a set of tests was made by one of the authors on slabs of 3-foot and 6-foot span length.[1] These tests gave some useful and rather surprising results that have since been

[1] Goldbeck, A. T. Tests of reinforced-concrete slabs under concentrated loading. *In* Amer. Soc. Testing Materials, Proc. 16th Ann. Meeting 1913, v. 13, p. 858–873, 10 fig. 1913. Discussion, p. 874–883, 4 fig.

verified; and in order to carry the investigation farther, with slabs of longer span than those previously investigated, the present series of tests was undertaken at the Arlington Experimental Farm of the United States Department of Agriculture.

## OBJECT OF INVESTIGATIONS

The theory applied to the design of narrow rectangular reinforced-concrete beams involves the assumption that the stress is constant throughout the width of the beam. In a wide slab the stress distribution varies from a maximum at the point of application of the load to a minimum at the extreme edges. Obviously then, if the rectangular-beam theory were applied to the design of slabs under concentrated loads, the width $b$ used in the design formulas can not be taken as the entire width of the slab. The rectangular-beam theory, however, could be utilized in wide-slab design if it were known what width $b$ should be substituted in the design formulas, and it is the object of this paper to explain tests for determining this width and to demonstrate the application of the theory of narrow rectangular beams to the design of wide slabs supported at two ends and subjected to concentrated loads.

## EFFECTIVE WIDTH

The width of the slab that should be used in the rectangular-beam formulas when applied to slab design will be termed the "effective width" of the slab. It is that width over which, if the stress were constant and equal to the maximum stress under actual conditions, the resisting moment would equal the resisting moment of a slab of the same depth and full width, but having varying stress distribution. If the straight-line theory of stress distribution from neutral axis to upper fibers is assumed to be applicable to slabs, the resisting moment of a given slab is dependent on the total stress in the concrete or steel at the dangerous section. The total stress in the concrete, however, is governed by the stresses in the top fibers, and these stresses are proportional to the unit deformations. If, then, there are two slabs of equal depth, one having uniform distribution of deformations and the other a varying distribution, but with their maximum deformations identical, they will likewise have equal resisting moments if the summations of the deformations over their respective widths are identical.

In figure 1, which represents a slab in position on two supports with a concentrated load $P$, is illustrated the method of obtaining "effective width." Strain-gauge readings are taken of the fiber deformations perpendicular to the supports, as indicated at *eg*. These concrete deformation values are plotted to scale, as, for instance, at *fh*, giving the deformation curve *JHF*, inclosing the area *AJHFE*. This curve shows the variation of stress from the center to each of the two free edges of the slab, and the area under the curve is a function of the total concrete-resisting

moment of the slab. The area *BDGI*, obtained by dividing the area *AJHFE* by its maximum ordinate *CH*, has the same total concrete-resisting moment with the stress uniformly distributed as the whole slab, and its width *BD* is that which may be effective in furnishing sufficient resistance under these conditions to carry the load. The width *BD*, obtained in this manner, is the "effective width."

## DESCRIPTION OF APPARATUS

LOAD-APPLYING APPARATUS.—The slabs tested were 32 feet wide, with a span length of 16 feet, and in order to accommodate such extraordinarily large test specimens it was necessary to build special apparatus. Two supports 32 feet long were constructed of reinforced concrete, and embedded in each of them at the center were two loop-welded eyes carrying four 24-inch 80-pound I beams 6 feet above the level of the supports

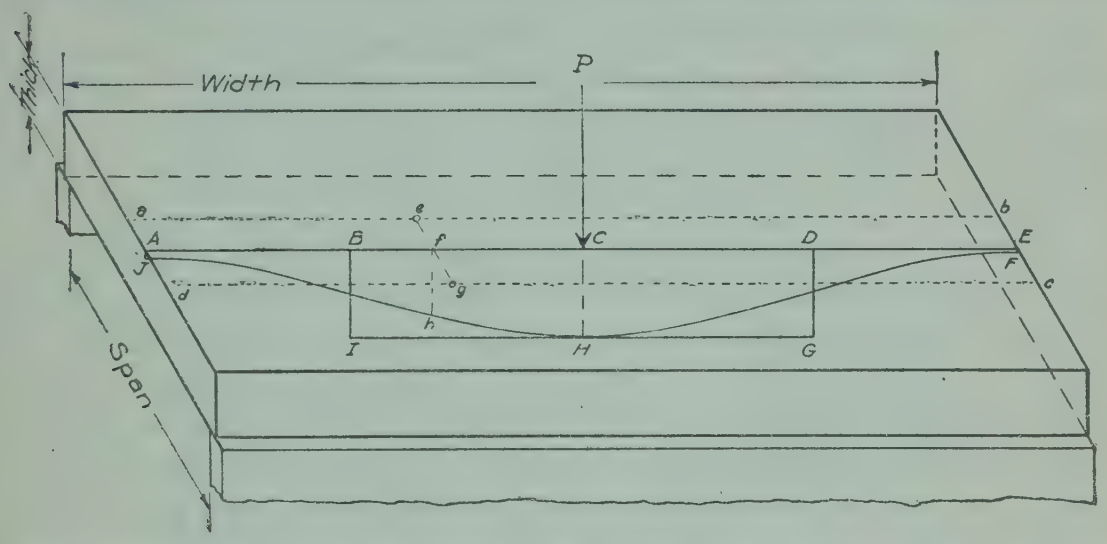



FIG. 1.—Diagram illustrating the method of obtaining "effective width" in reinforced-concrete slab tests.

(Pl. XXVI). Loads were applied by means of a hand-operated hydraulic jack mounted between the slab and the overhead I beams.

For weighing the loads a specially calibrated chrome-nickel beam (Pl. XXVI) was mounted between the jack and the load-applying I beams, and its deflection at the center was a measure of the load applied. This chrome-nickel beam was 7 inches wide, 5 inches deep, and 27 inches in span, and its deflection was measured with an Ames dial reading to 0.0001 inch. The dial was fastened to the beam and its plunger rested on a ½-inch square steel rod mounted on the side of the beam at the neutral axis. It was found that by fastening an electric buzzer on this rod more consistent readings could be obtained with the dial. The entire load-applying device was calibrated in a 200,000-pound universal testing machine, and the beam deflections corresponding to known loads were obtained. A deflection of approximately 0.0001 inch occurred for each 500 pounds of load applied. A number of calibrations were made and a calibration curve was plotted. When used for measuring loads, it was only necessary to read the central deflection on the Ames dial and the corresponding load could be read from the curve.

DEFORMATION-MEASURING APPARATUS.—Deformations of the top of the slab were measured at right angles to the supports, and also, in the case of one slab, parallel to the supports, with a Berry strain gauge of 20-inch gauge length. The degree of accuracy attained was probably within 0.0002 inch in that gauge length. Short brass plugs drilled at one end with a No. 55 drill were embedded in the concrete, or in some cases cemented in holes drilled for the purpose; and the movements of these plugs as measured with the strain gauge were considered the fiber deformations.

In the last slab tested (No. 934) deformation readings were also taken of the steel reinforcement, and for this purpose holes were drilled in the steel bars 20 inches apart to accommodate the points of the strain gauge. Although readings were not taken on all of the bars, a sufficient number were measured to determine the distribution of the steel stresses throughout the slab. The layout of strain-gauge points between which readings were made is shown in figures 2, 3, and 4. The arrowheads mark the position of the points on the top of the slab and in the case of slab 934 (fig. 4) the gauge points in the steel are marked by small circles.

DEFLECTION-MEASURING APPARATUS.—The deflection measurements were made in somewhat different ways during these tests, and the apparatus was improved as the tests progressed. In its final form in slab 934, the deflection-measuring equipment consisted of a network of piano wires stretched tightly at a fixed distance above the concrete supports, and being entirely independent of the slab. At the points where measurements were taken, steel plates were set in plaster of Paris on top of the slab. Readings were then made between these plates and the wires by means of a specially designed instrument consisting of a brass stand carrying a bell-crank lever, one end of which touched on the piano wire above and the other end bore on the plunger of an Ames dial. By means of a slow-motion screw the end of the bell-crank lever was adjusted to touch the wire as indicated by an electric buzzer. The dial readings taken at different loads then indicated the deflections at the various points on the slab. This instrument is probably a more convenient form of measuring device than the ordinary inside micrometer and is accurate to 0.004 inch.

## DESCRIPTION OF SPECIMENS

All three specimens were 32 feet wide, 16 feet span, and were made of machine-mixed concrete in the proportions 1 to 2 to 4. Potomac River sand and gravel were used as the aggregates, mixed with Portland cement. A rather wet mix was used, and the work of molding was done by laborers at the Arlington Farm who were experienced in work of this character. There was no attempt to make the concrete any better than it would ordinarily be made in the field, but efforts were

directed to secure work thoroughly representative of that obtained under field conditions. The sand was a good grade for use in concrete, and the gravel was clean, well graded, and free from weak pebbles.

The steel reinforcing consisted of ¾-inch plain square bars in slabs 835 and 930, and the bars in slab 934 were ½-inch square. The yield point of this material is about 39,000 pounds, and the ultimate strength 60,000 pounds per square inch.

The slabs were necessarily built in place on their supports, and the forms were struck at the end of about two weeks. The concrete was sprinkled daily for several weeks during the earlier stages of hardening and was allowed to cure protected from the weather until the destruction of the slab.

Table I contains the essential data concerning the slabs tested.

TABLE I.—*Description of reinforced-concrete slabs used in tests*[1]

| Serial No. | Thickness. | | Reinforcing. | | | Modulus of elasticity of concrete. | Central breaking load of slab. |
|---|---|---|---|---|---|---|---|
| | Total. | Effective. | Size. | Spacing. | Per cent. | | |
| | *Inches.* | *Inches.* | *Inches.* | *Inches.* | | | *Pounds.* |
| 835..... | 12 | 10½ | ¾ (plain square). | 10. 5 | 0. 75 | 2, 900, 000 | 119, 000 |
| 930..... | 10 | 8½ | ¾ (plain square). | 8. 87 | . 75 | 4, 000, 000 | 80, 000 |
| 934..... | 7 | 6 | ½ (plain square). | 5. 56 | . 75 | 3, 000, 000 | 40, 000 |

[1] The slabs were not reinforced transversely.

At the time the slab specimens were made, 8 by 16 inch concrete cylinders were molded from the same mixture and were allowed to cure under the same conditions as the slabs. These were tested later for their crushing strength and modulus of elasticity.

## METHOD OF TESTING SLABS

At the age of 28 days the initial strain-gauge and deflection readings were taken with no load on the slab. The first load was then applied through an 8-inch cylindrical bearing block set in plaster of Paris at the center of the slab. Strain-gauge and deflection observations were made again over the entire slab. Due account was taken of the air and concrete temperatures in order to make corrections for any appreciable change occurring during the progress of the tests. The increments of load applied to the different specimens were varied in the different slabs, depending on their thickness, and the aim was to stress neither the steel nor the concrete beyond working limits, also to obtain about five increments of load within the working load.

After readings over the entire slab had been taken, check readings were made at various points; and invariably it was found that these check readings showed an increased deformation in the concrete even though its temperature remained constant. Moreover, upon releasing the load entirely it was found that considerable permanent deformation remained in the concrete. This phenomenon can be attributed only to the "flow" or gradual change in length of the concrete even when under small stresses and is significant, for it shows the importance of the time effect on the relation of stresses and strains in concrete. If the strain readings on the top of the slab, loaded for five or six hours, be used to estimate the stresses in the concrete, based on the initial modulus of elasticity of the concrete, this estimated stress will be greatly in excess of the true stress conditions.

In view of the fact that the deformations which take place in the concrete under a sustained load are continually increasing and remain partially permanent, and that the only deformations of value are those indicative of the stress, all of the final calculations and deductions are based upon results obtained by taking zero deformation readings just before applying the load. Deformations thus obtained by taking the difference between the strain-gauge readings at the zero load and the testing load (all within an hour or so), represent more accurately the elastic deformations and are a better indication of the stress existing in the concrete than those obtained from any initial or previous zero readings.

## GRAPHICAL REPRESENTATION OF DATA AND RESULTS

A great amount of numerical data has been taken during the tests of these three concrete slabs. Some of these data were preliminary and served only to indicate methods and limits. Those data which have a direct bearing upon the problem are shown graphically in the accompanying curves (fig. 2–28).

FIGURES 2, 3, AND 4.—The layout of the points in the concrete and the steel over which the strain-gauge readings were taken are shown in figures 2, 3, and 4. In a few cases readings were made between all points, but in general only the readings along a center line (5–6) parallel to the supports were taken, as this gives sufficient data for determining the effective width. In all mention of strain-gauge or deformation readings it should be understood that they are measured between points on a line perpendicular to the supports, unless expressly stated to be otherwise.

FIGURE 5.—Figure 5 shows the variation of the concrete deformations for different concentrated center loads, along the center line of the slab. The ordinates of these curves are influenced slightly by the time factor or "flow" in the concrete; hence, the values for the effective width $b$ are somewhat erratic in their relation to the load.

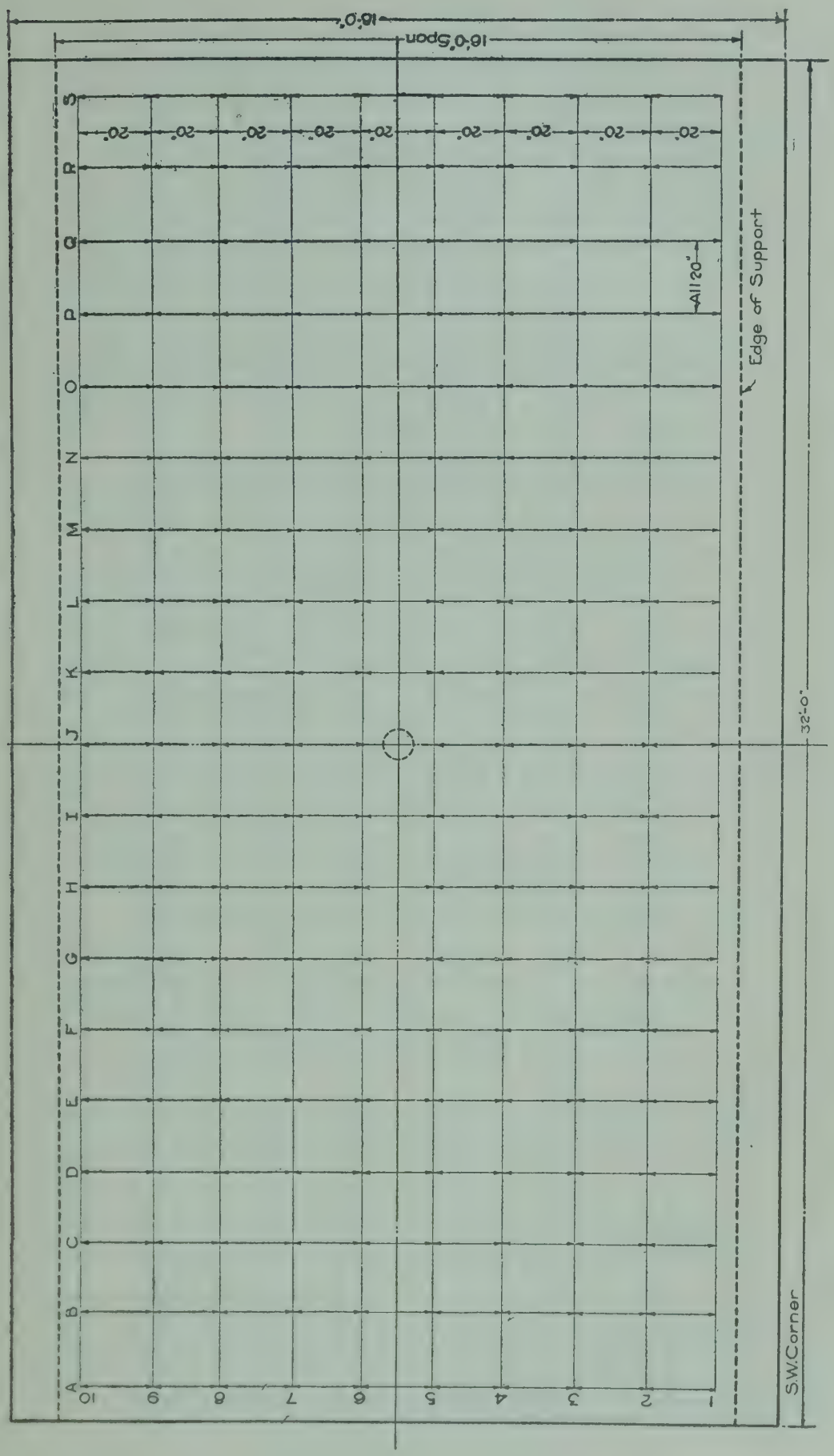



FIG. 2.—Diagram showing location of strain-gauge points on top of slab 835.

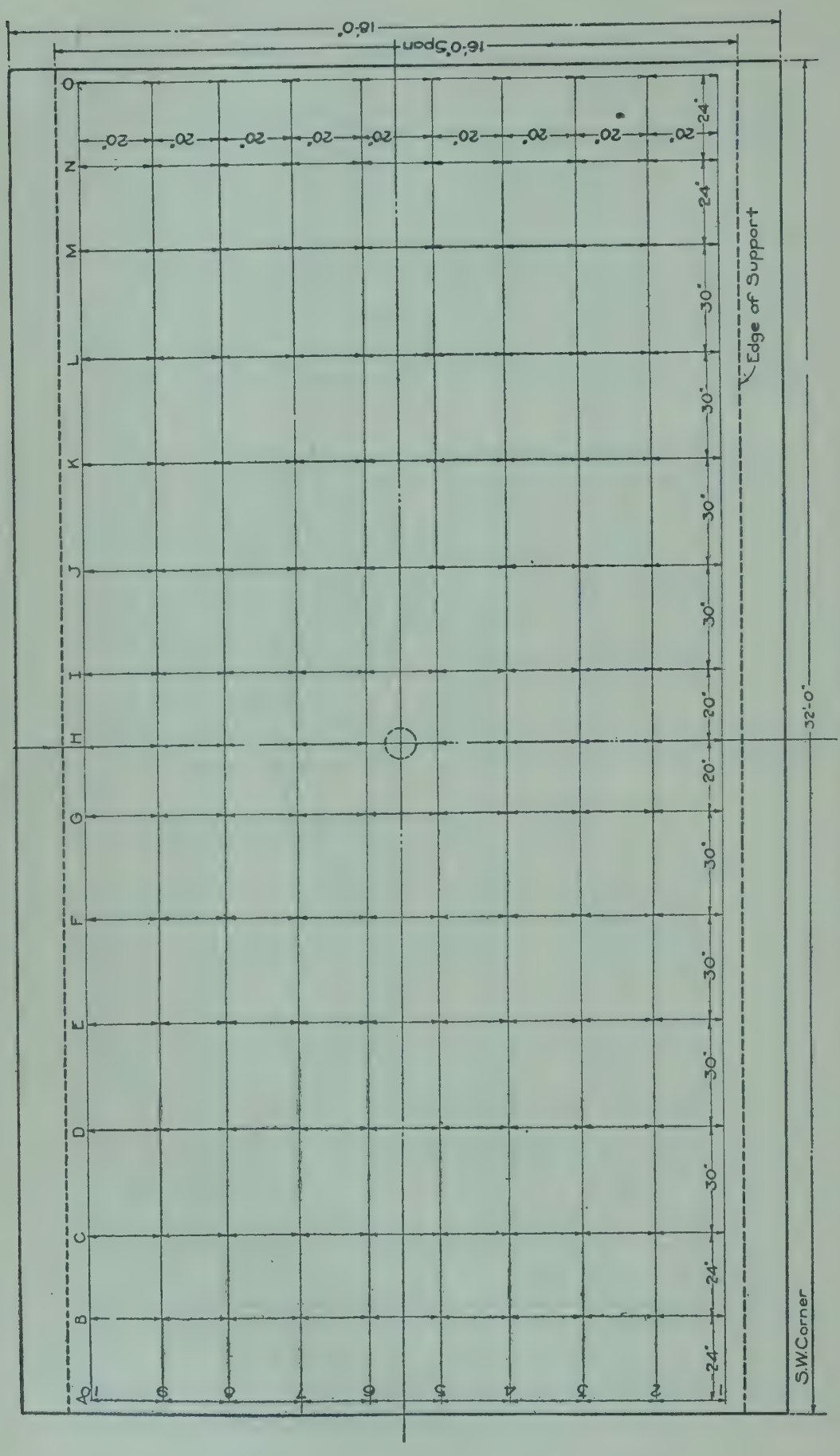



FIG. 3.—Diagram showing location of strain-gauge points on top of slab 930.

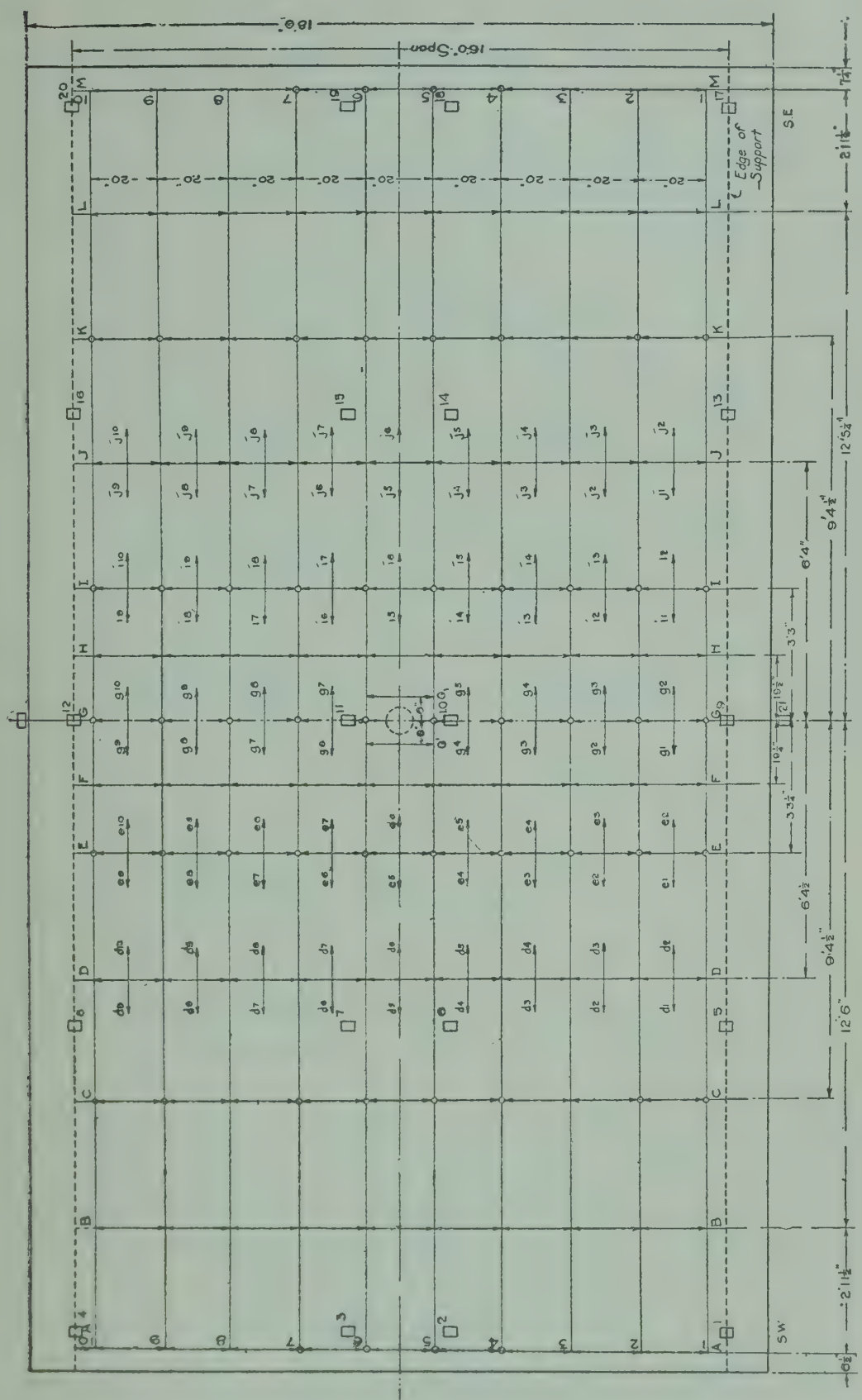



FIG. 4.—Diagram showing location of strain-gauge points on top and bottom of slab 934.

FIGURE 6.—Two curves, A and B, are shown here to indicate the deformations which resulted from the removal of the forms. The flow, or increase in the deformations, is about 80 per cent in three days. The curves C–D, E–F, G–H, I–J, and K–L show the large difference in the deformation and effective width values between those obtained by the use of a zero strain-gauge reading taken several weeks before, with several intervening loadings, and those obtained from a zero reading taken just before the loading. The data and results of curves C, E, G, I, and K are the only ones of value.

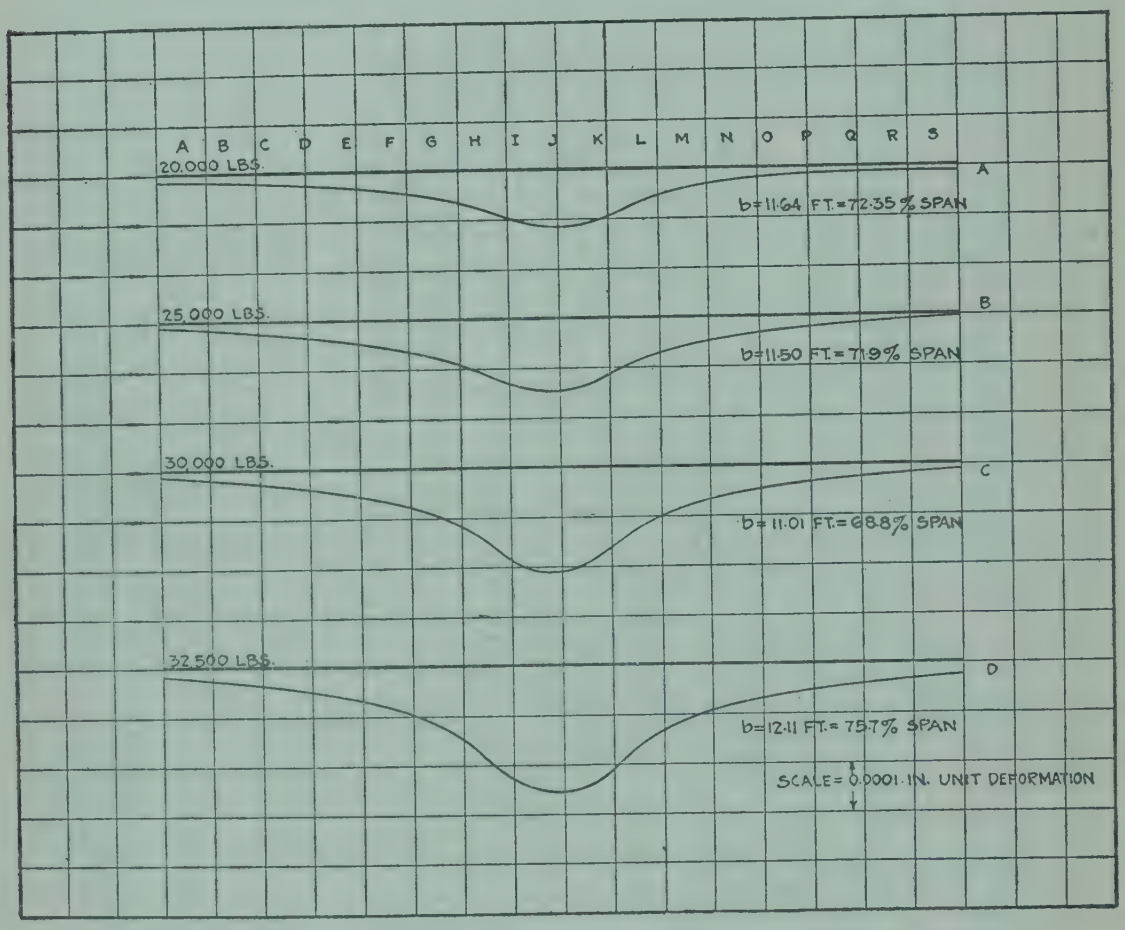



FIG. 5.—Concrete deformation curves for concentrated center load on slab 835.

FIGURE 7.—The difference between these curves shows the magnitude of the set, or permanent deformation, which may occur between two applications of the load, each loading having been applied immediately after a zero reading of the strain-gauge points, with 24 hours intervening between the loadings. The second application shows a smaller deformation than the first. This is true for both the concrete and the steel deformations. The effective widths are based upon the first application of the load.

FIGURE 8.—These curves are shown to emphasize the importance of considering the time factor and its effect upon the deformations in concrete structures. Curve 1 shows the immediate effect of the load. After about 5 hours the load was removed, then again applied 20 hours later

and allowed to remain on for two days, giving curves 2 and 3. The load was then removed, and curve 4 shows the amount of set about two hours later. This set is somewhat reduced after a few days' rest. The values of the effective widths shown in this figure differ very largely and are also indicative of the fact that the time factor is very important.

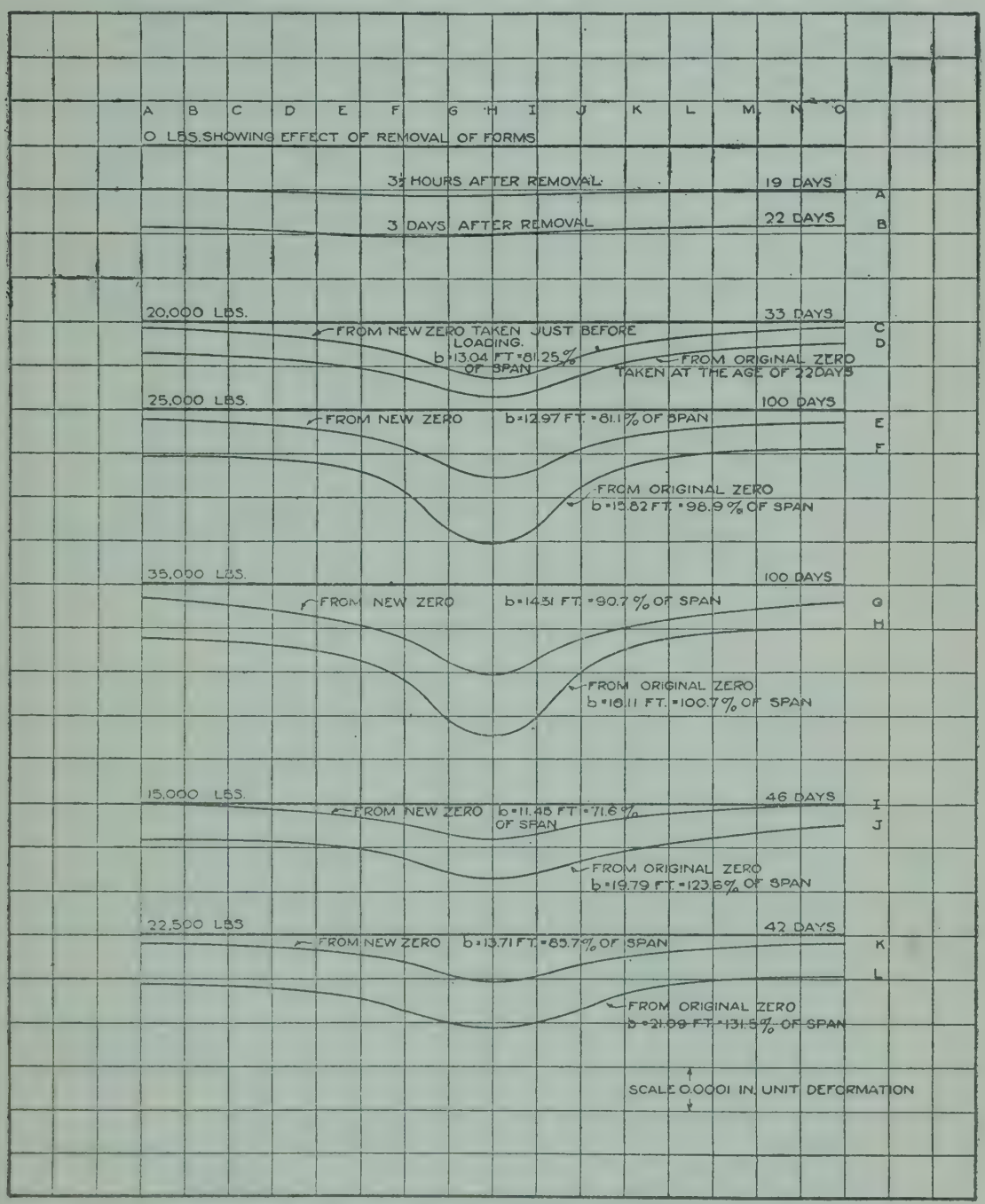



FIG. 6.—Concrete deformation curves for slab 930.

FIGURE 9.—Concrete deformations under 2-point loadings are shown for two-load values. The 40,000-pound load was applied immediately after taking the zero reading, and the deformations taken at once. The load was then increased to the 80,000-pound value and deformations again taken. The whole operation required not over two hours. The local effect at the load points is very pronounced for the larger load.

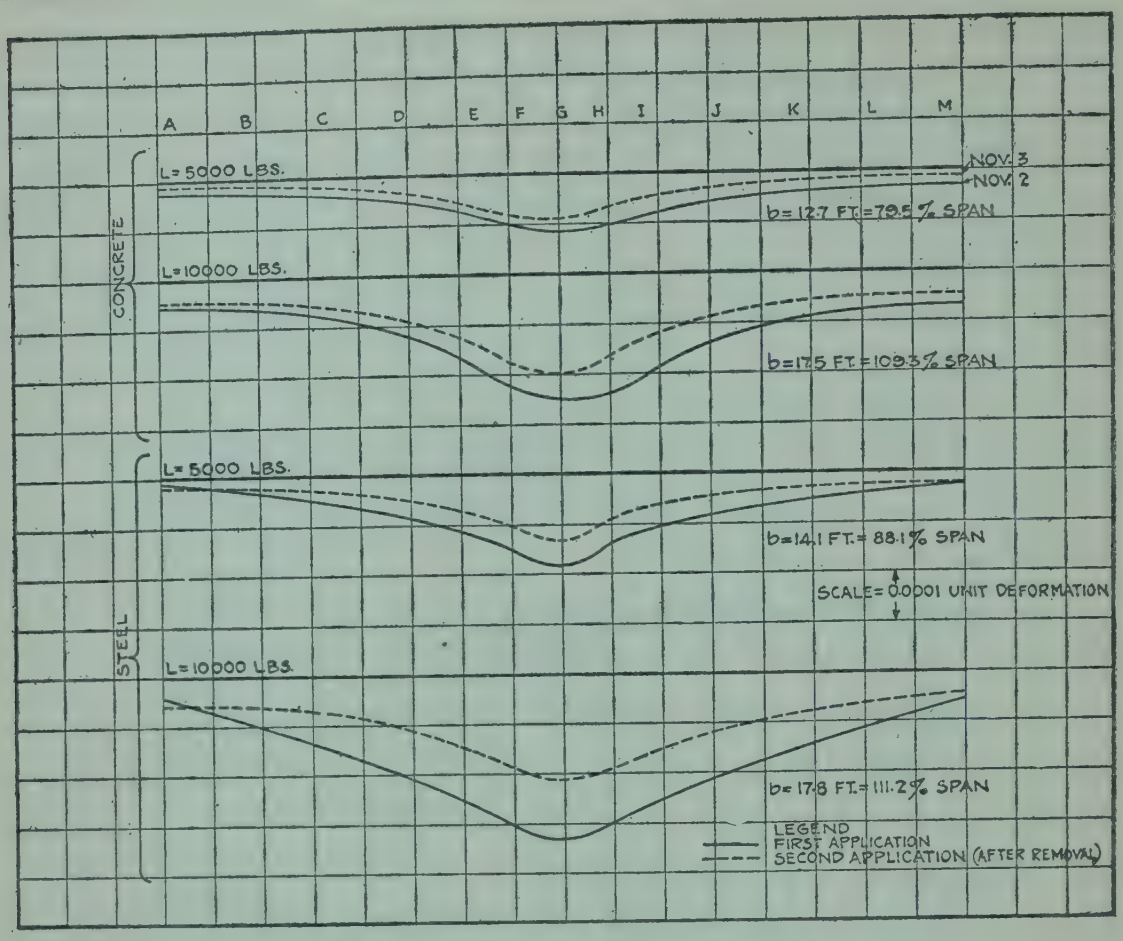



FIG 7.—Deformation curves for slab 934.

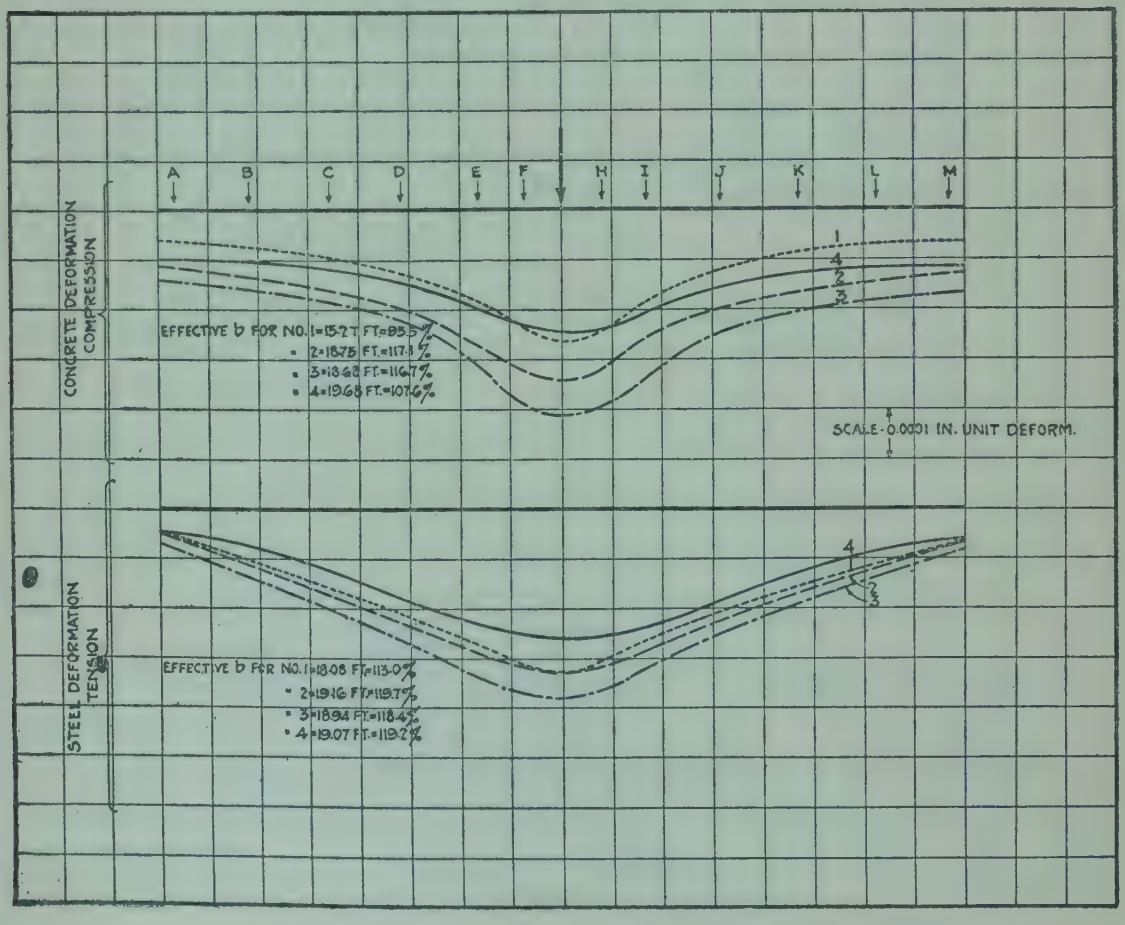

A B C D E F H I J K L M
CONCRETE DEFORMATION COMPRESSION
EFFECTIVE b FOR NO. 1=15.2 FT.=85.5 %
2=18.75 FT.=117.1 %
3=18.42 FT.=116.7 %
4=19.68 FT.=107.6 %
SCALE-0.0001 IN. UNIT DEFORM.
STEEL DEFORMATION TENSION
EFFECTIVE b FOR NO. 1=18.08 FT.=113.0 %
2=19.16 FT.=119.7 %
3=18.94 FT.=118.4 %
4=19.07 FT.=119.2 %

FIG. 8.—Deformation curves for slab 934, computed from first zero reading.

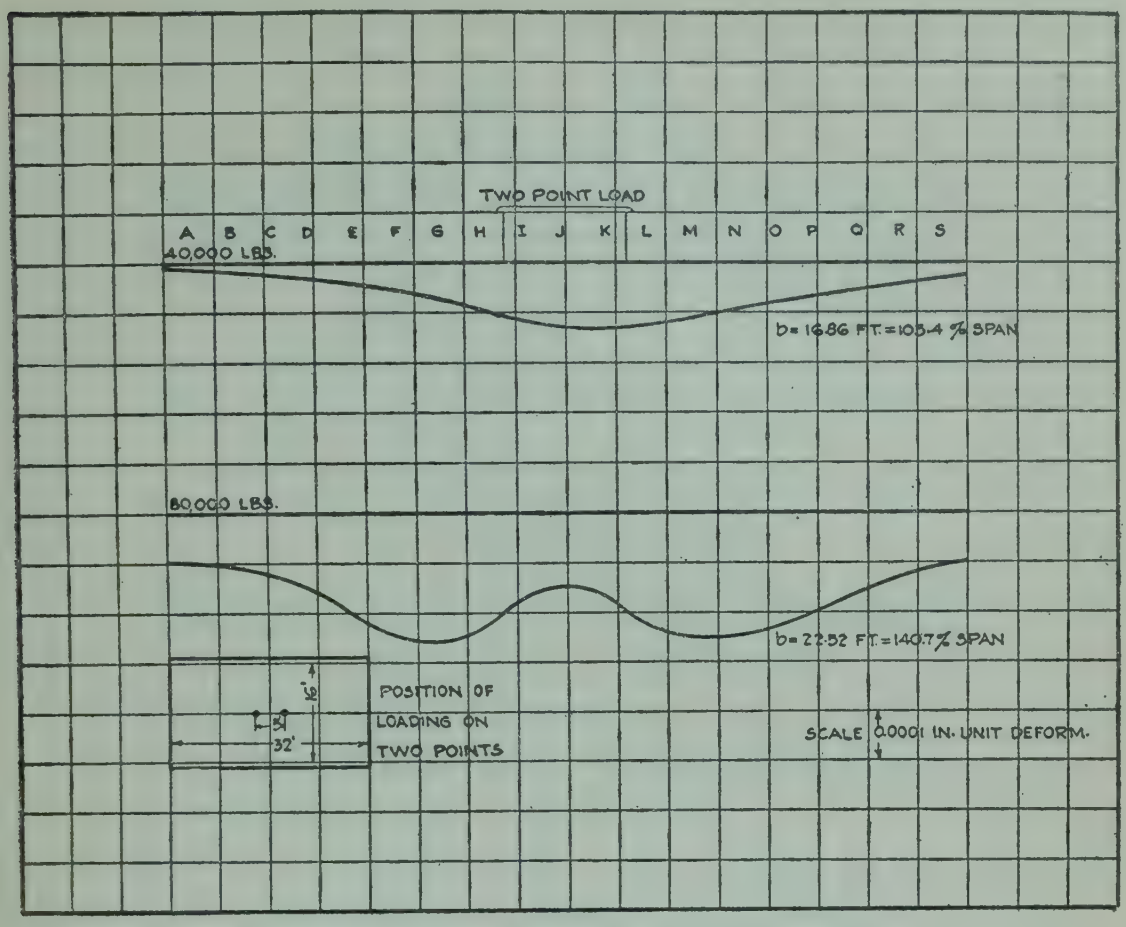

TWO POINT LOAD
A B C D E F G H I J K L M N O P Q R S
40,000 LBS.
b = 16.86 FT. = 105.4 % SPAN
80,000 LBS.
b = 22.52 FT. = 140.7 % SPAN
POSITION OF LOADING ON TWO POINTS
32'
SCALE 0.0001 IN. UNIT DEFORM.

FIG. 9.—Concrete deformation curves for slab 835 with 2-point loading.

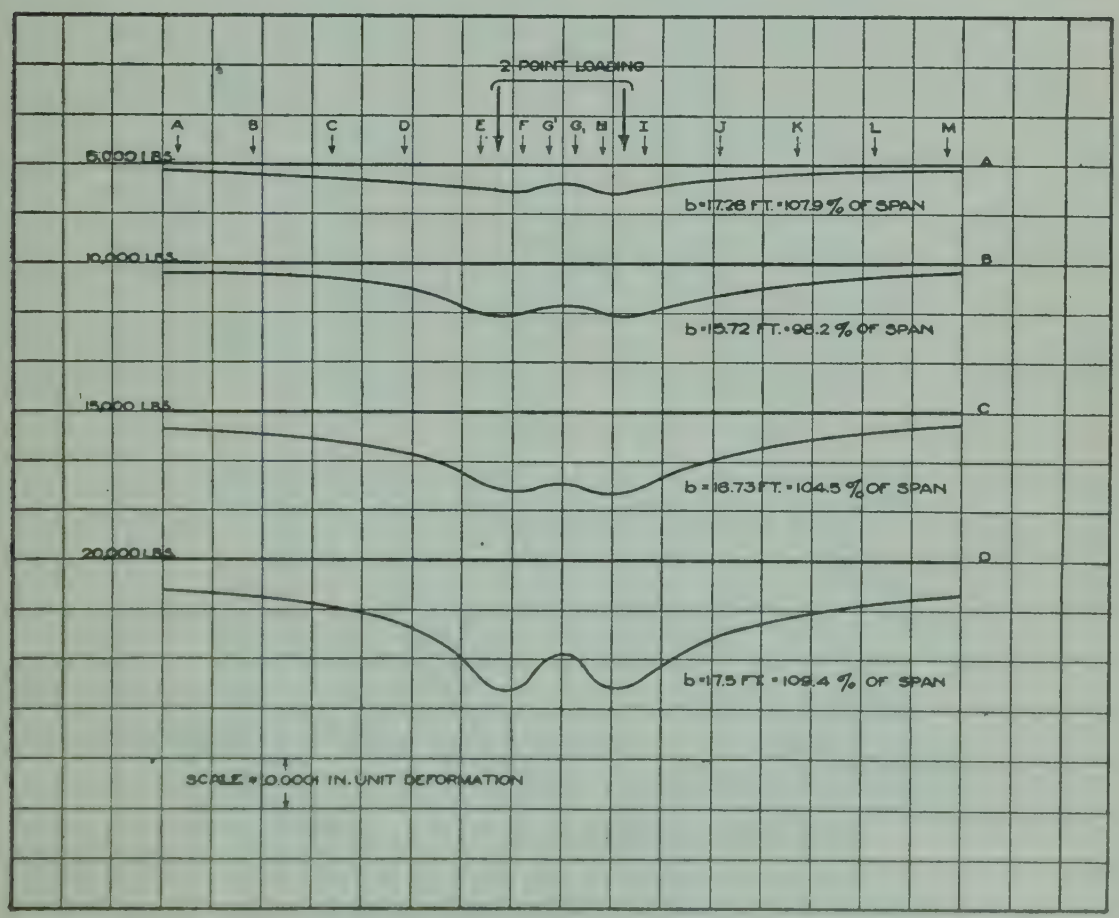

2-POINT LOADING
A B C D E F G' G, H I J K L M
5,000 LBS.
A
b = 17.28 FT. = 107.9 % OF SPAN
10,000 LBS.
B
b = 15.72 FT. = 98.2 % OF SPAN
15,000 LBS.
C
b = 16.73 FT. = 104.5 % OF SPAN
20,000 LBS.
D
b = 17.5 FT. = 109.4 % OF SPAN
SCALE = 0.0001 IN. UNIT DEFORMATION

FIG. 10.—Concrete deformation curves for slab 934 with 2-point loading.

The effective width is not materially affected for the 40,000-pound load; but for the 80,000-pound load, which produces the working fiber stress, the effective width is very largely increased.

FIGURES 10 AND 11.—The curves on these figures show a more pronounced local effect in the concrete at the load points than the same character of loading on the thicker slab. It should be noted that for the working load of 20,000 pounds the effective width for this 2-point loading is the same as for the single-point center loading.

FIGURES 12 AND 13.—The results for 4-point loading under different loads are shown in these curves for slabs 835 and 934. The effective

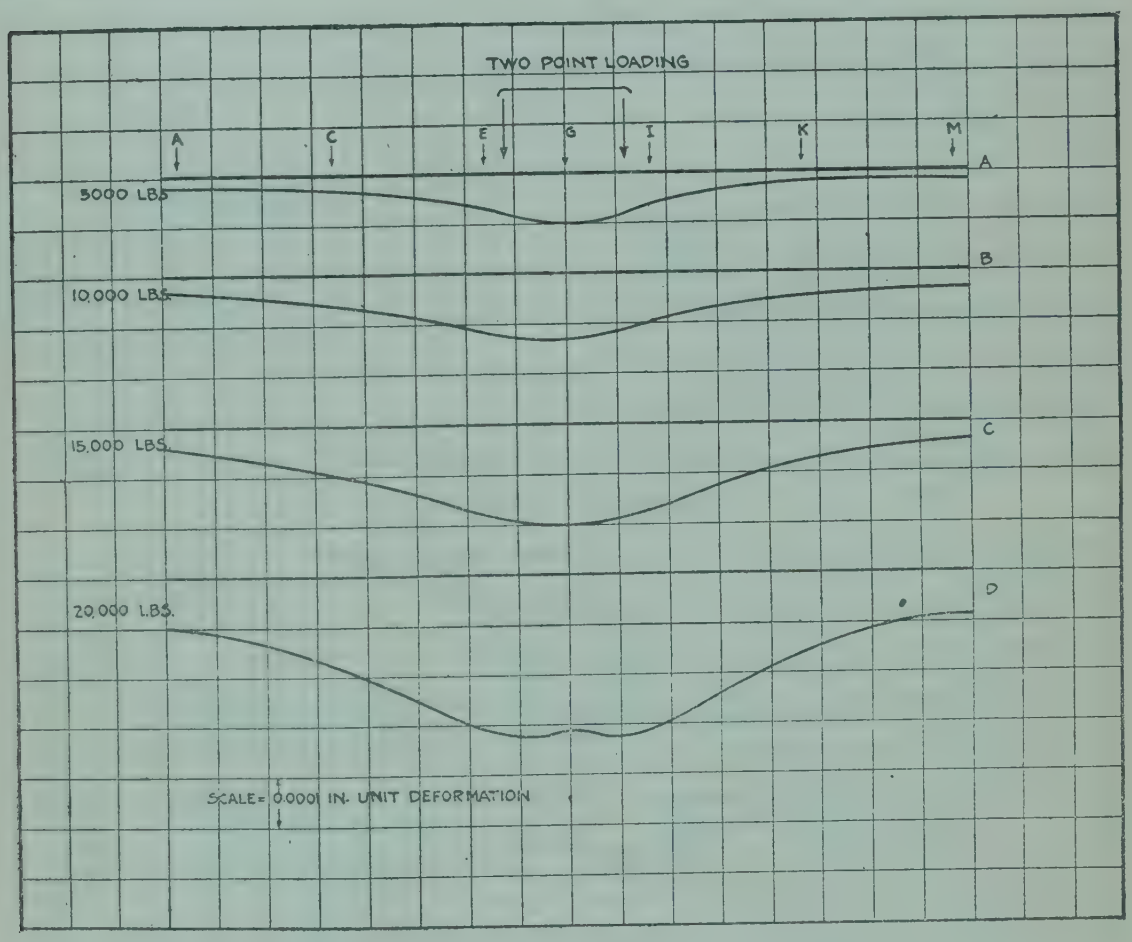



FIG. 11.—Steel deformation curves for slab 934 with 2-point loading.

width is materially affected by the width between the load points; it seems to be increased by not less than 56 per cent of the span length for slab 835, and 93 per cent for slab 934.

FIGURES 14, 15, AND 16.—The deflection data are shown on these figures. The curves are plotted to show the deflection values along a center strip parallel to the supports. In figure 14 curves have been plotted showing the flow and set in the slab under a sustained load and as effected by two applications. Two values for effective widths are shown, which have been obtained from the deflection curves in the same manner as from the concrete deformation curves described above; but these values should not be used in the design of slabs.

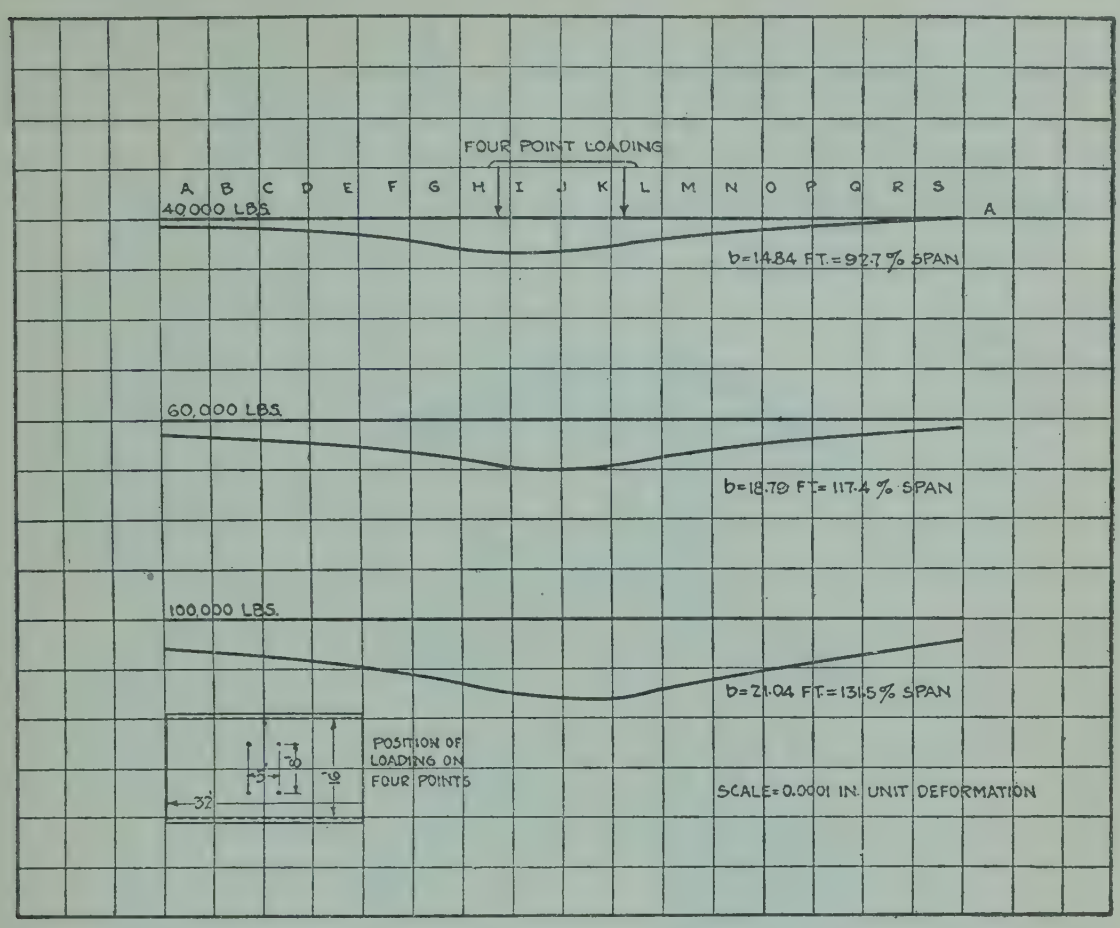

FOUR POINT LOADING
A B C D E F G H I J K L M N O P Q R S A
40000 LBS.
b=14.84 FT.=92.7% SPAN
60,000 LBS.
b=18.78 FT.=117.4% SPAN
100,000 LBS.
b=21.04 FT.=131.5% SPAN
POSITION OF LOADING ON FOUR POINTS
SCALE=0.0001 IN. UNIT DEFORMATION

FIG. 12.—Concrete deformation curves for slab 835 with 4-point loading.

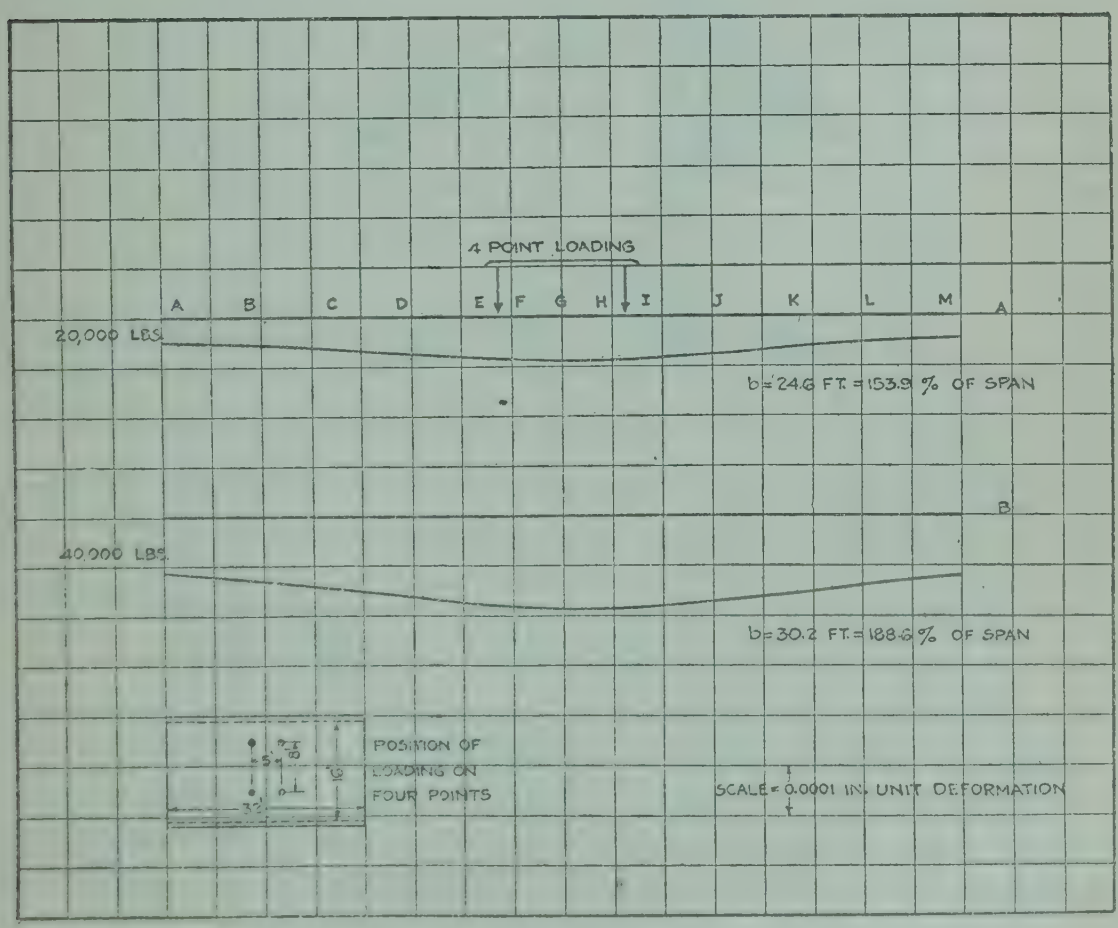

4 POINT LOADING
A B C D E F G H I J K L M A
20,000 LBS.
b=24.6 FT.=153.9% OF SPAN
B
40,000 LBS.
b=30.2 FT.=188.6% OF SPAN
POSITION OF LOADING ON FOUR POINTS
SCALE=0.0001 IN. UNIT DEFORMATION

FIG. 13.—Concrete deformation curves for slab 934 with 4-point loading.

FIGURES 17, 18, AND 19.—After each slab was broken the cracks in the top and bottom were drawn to scale. The heavy full lines forming an approximate circle or ellipse around the load point are the tension cracks on the top of the slab caused by the overhang of the ends, after a large center deflection, at about breaking load. The remarkable symmetry of

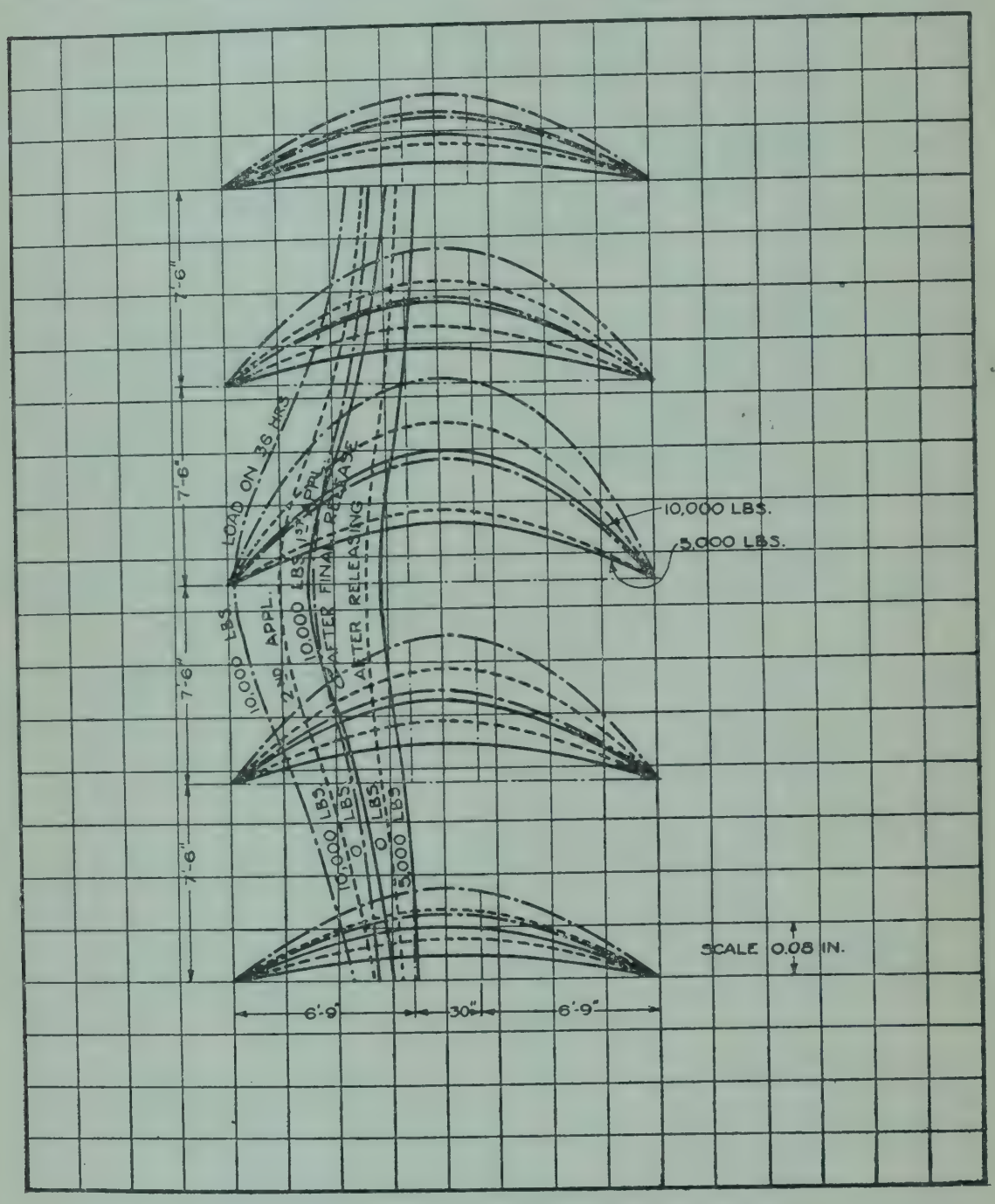



FIG. 14.—Deflection curves for slab 934 on first application of load.

these cracks is worthy of notice. There seems to be no definite relation between the effective width at working loads and the width over which the cracks extended at failure; in fact, it is hardly reasonable that there should be any definite relation, for one case is dealing with safe working stresses within the limit of elasticity, and the other with breaking loads.

Table II shows the breaking loads and their relation to the depth of the slab. Note that the breaking loads are almost directly proportional to the squares of the depths.

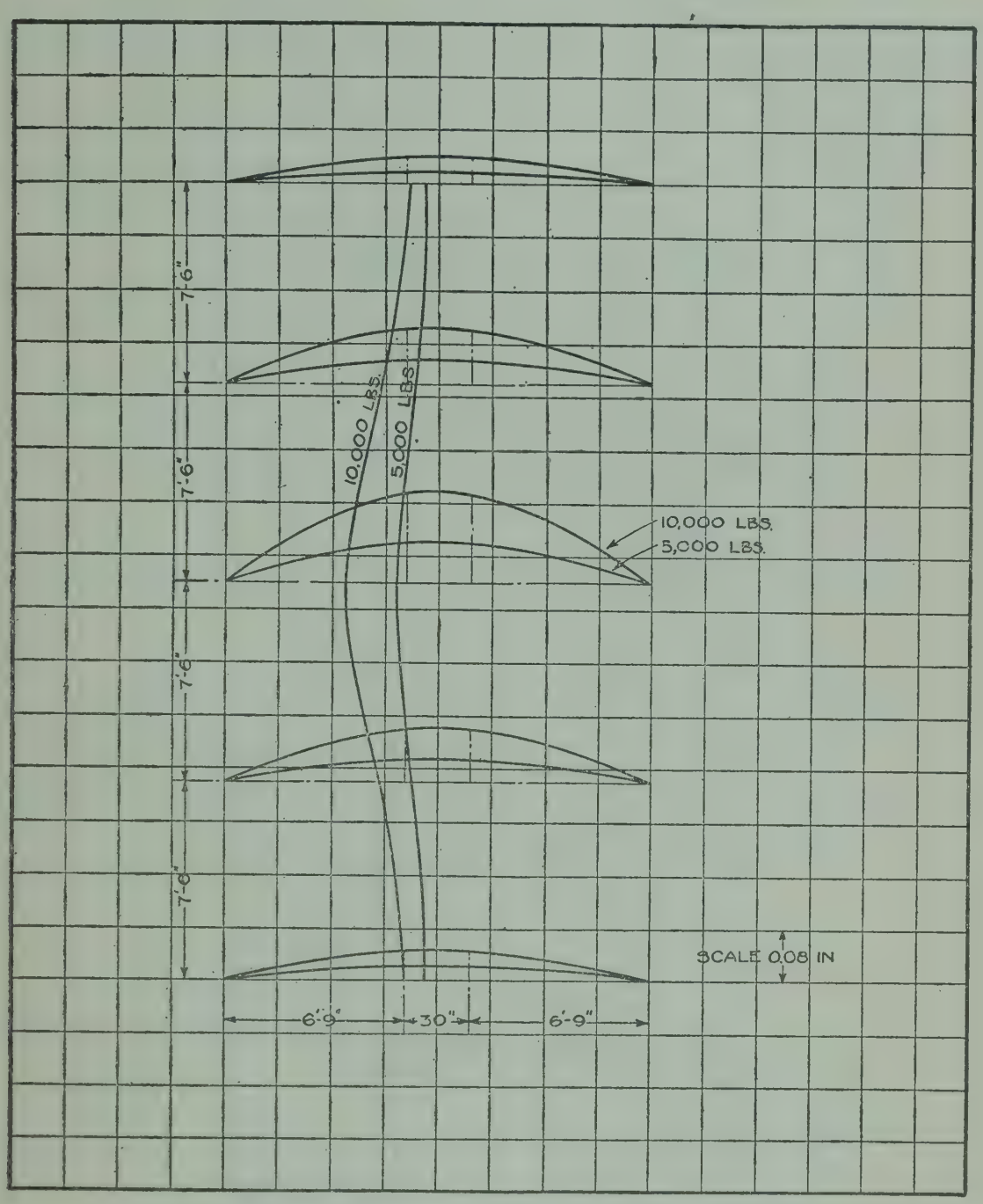



FIG. 15.—Deflection curves for slab 934 on second application of load.

TABLE II.—*Breaking loads of reinforced-concrete slabs and their relation to the depth of slab*

| Serial No. | Effective thickness, *d*. | $d^2$ | Breaking load. | Relations. | |
|---|---|---|---|---|---|
| | | | | $d^2$ | Loads. |
| 835 | 10½ | 110.25 | 119,000 | 3.06 | 2.98 |
| 930 | 8½ | 72.25 | 80,000 | 2.01 | 2.00 |
| 934 | 6 | 36.00 | 40,000 | 1.00 | 1.00 |

## STRESS DISTRIBUTION OVER THE WHOLE SLAB

For the purpose of determining the distribution of stress over the top of the whole slab, deformation readings at right angles to each other were taken on slab 934 for a working load of 10,000 pounds concentrated at the center.

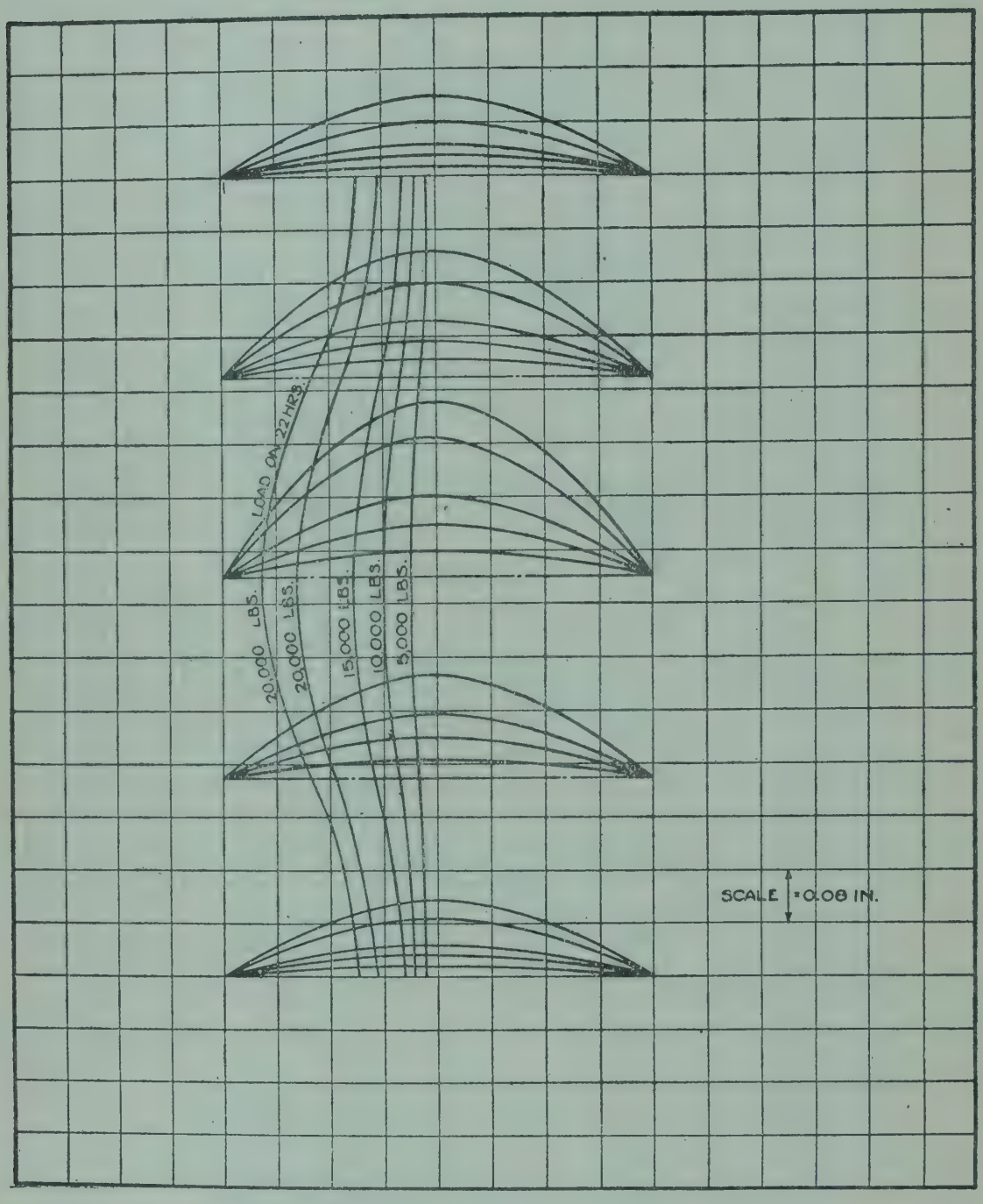



FIG. 16.—Deflection curves for slab 934 with 2-point loading.

FIGURES 20 AND 21.—The deformations measured perpendicular to the supports and plotted on base lines parallel to the supports are shown in figure 20. These curves show the variation of deformations along lines parallel to the supports. The same deformations plotted on base lines perpendicular to the supports, to show the variation in that direction,

are plotted on figure 21. Each curve as shown is an average of the plotted points. The light vertical lines serve only to locate each curve with its base line.

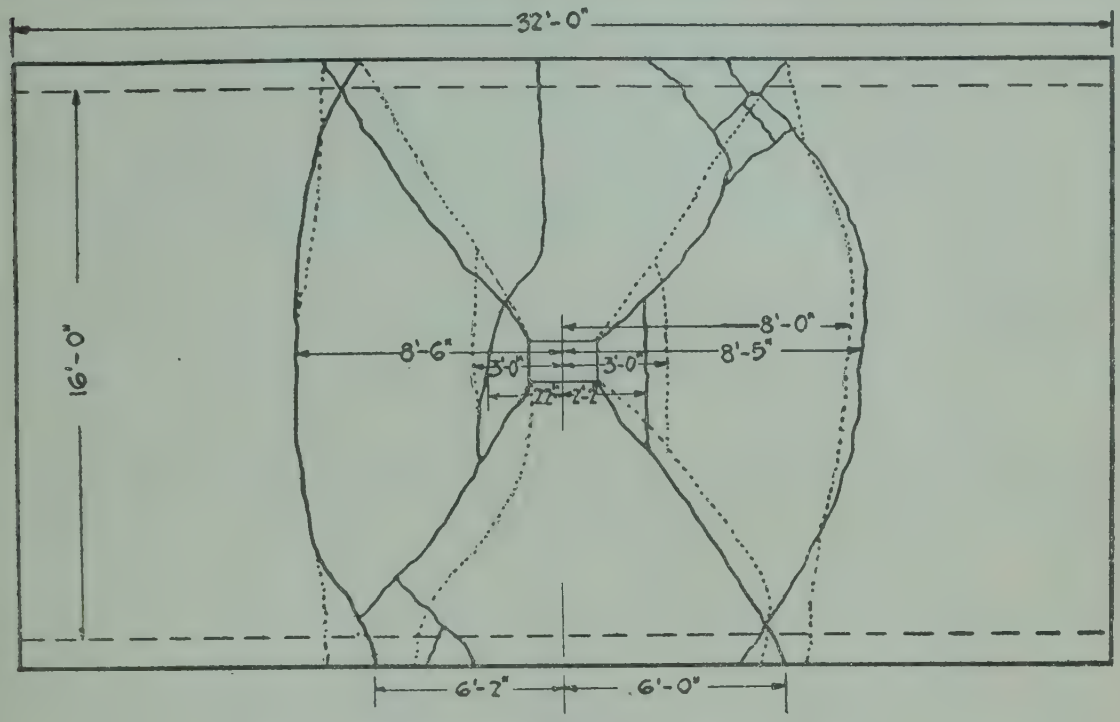



FIG. 17.—Diagram showing effect of breaking load on slab 835.

The variation of the distribution along lines parallel to the supports is is somewhat gradual and does not show any sudden changes; but the

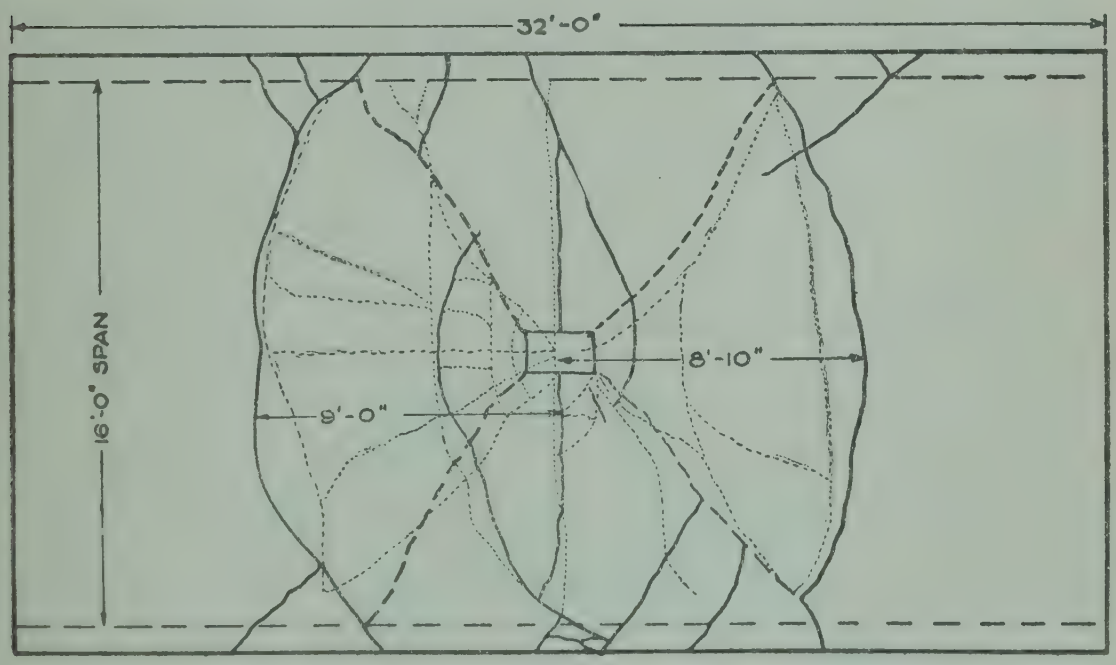



FIG. 18.—Diagram showing effect of breaking load on slab 930.

variation across the span near the center of the slab becomes somewhat critical at and near the load point, and this was more pronounced in the concrete than in the steel. (The steel data are not shown.)

FIGURE 22.—Lateral strain-gauge readings were taken on points parallel to the supports over the middle third of the slab, and these are plotted on base lines both parallel and perpendicular to the supports. The groups of closely drawn parallel lines serve only to connect each curve with its base line. Compression values of the deformations are plotted either to the left or below the base lines, and to the right or above, for values of tension in the concrete. The variations in these lateral deformations are the reverse of those of the longitudinal deformations shown in figures 20 and 21; they are more critical along lines parallel to the supports.

FIGURE 23.—The data of the last three figures have been collected and plotted as "iso-deformation lines," giving a series of lines or contours

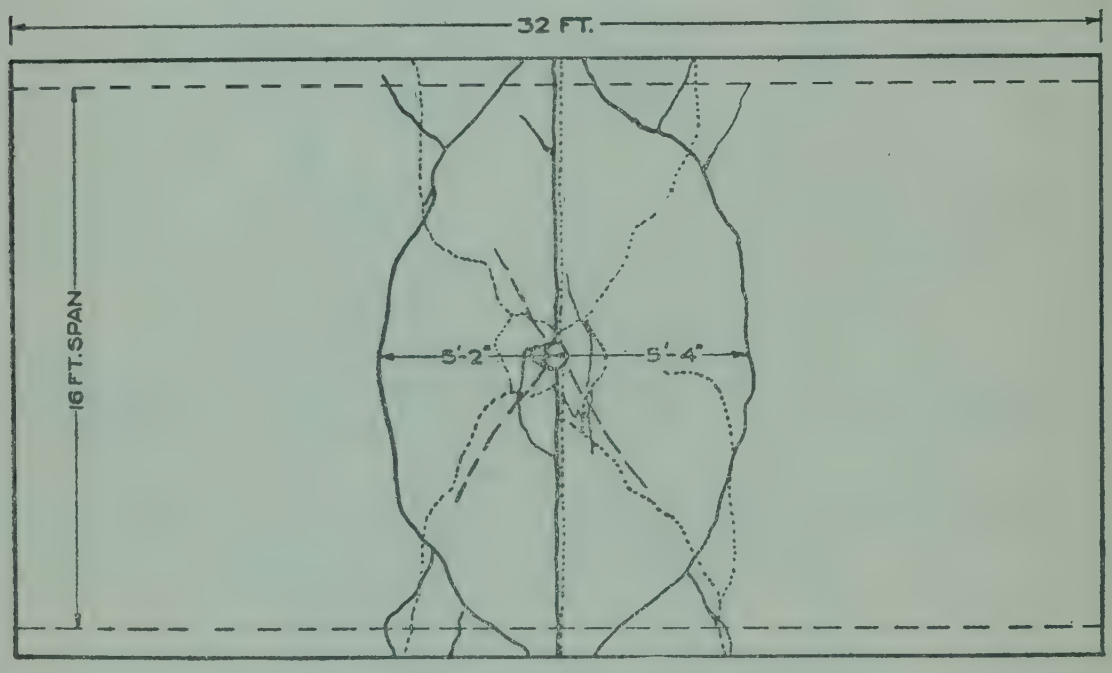



FIG. 19.—Diagram showing effect of breaking load on slab 934.

which represent equal deformations in the concrete on the top of the slab. The lines, as drawn, are averages of the plotted points. Figure 23 (also fig. 26) is more for academic interest and should be of service in the theoretical consideration of stress distribution.

FIGURES 24 AND 25.—These figures are similar to figures 20 and 21, and are plotted in the same manner, except that they represent the distribution of deformations under a working load of 40,000 pounds applied at four points. No lateral deformation readings are shown. The load points are indicated in figure 25. The local effect at the loading points is very pronounced.

FIGURE 26.—The data of the last two figures mentioned have been here collected and show the "iso-deformation lines" for the 4-point loading of 40,000 pounds, total. (See description of figure 23.)

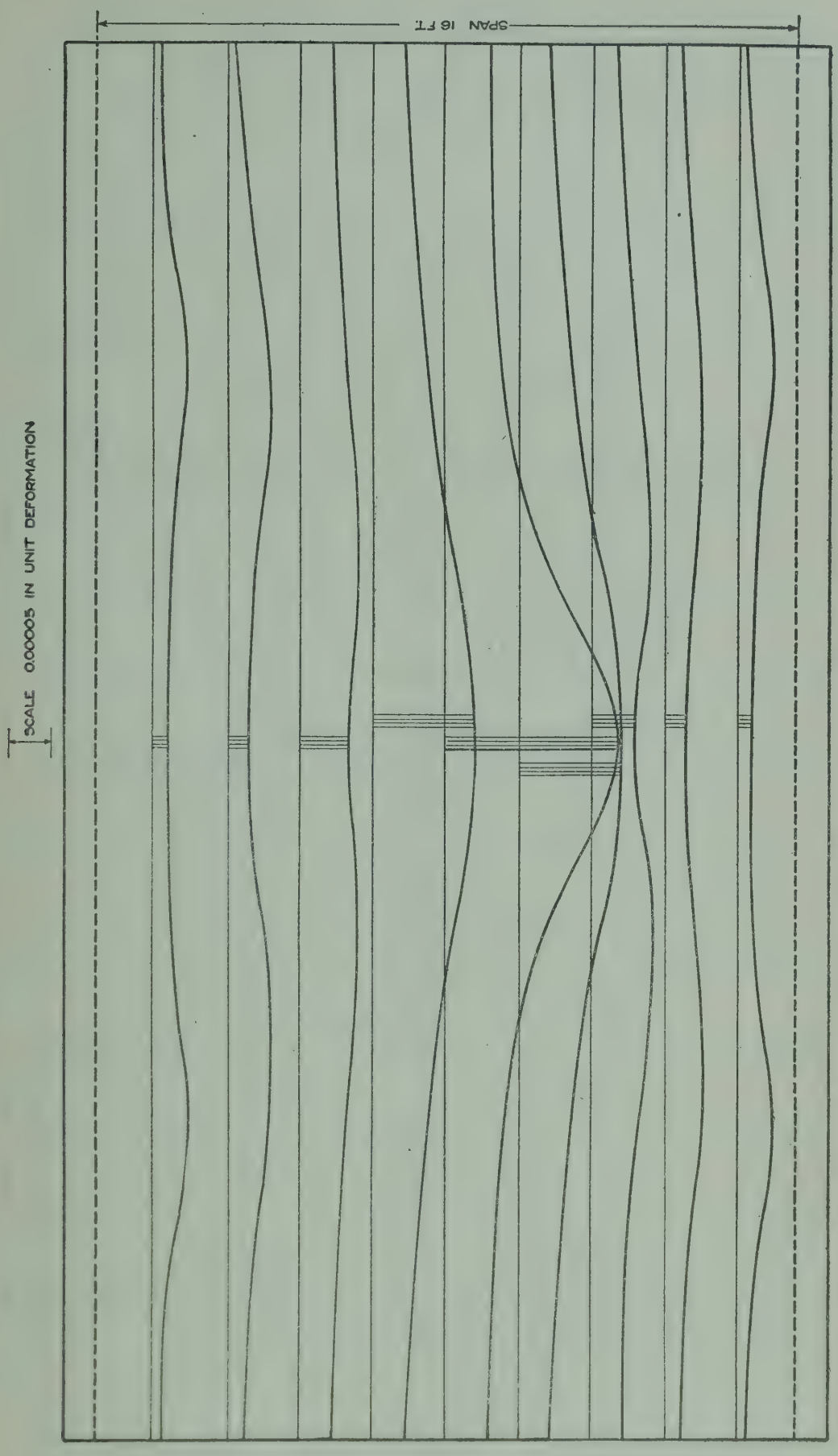



FIG. 20.—Concrete deformation curves for a 10,000-pound concentrated center load on slab 934. Variation in deformations plotted parallel to supports.

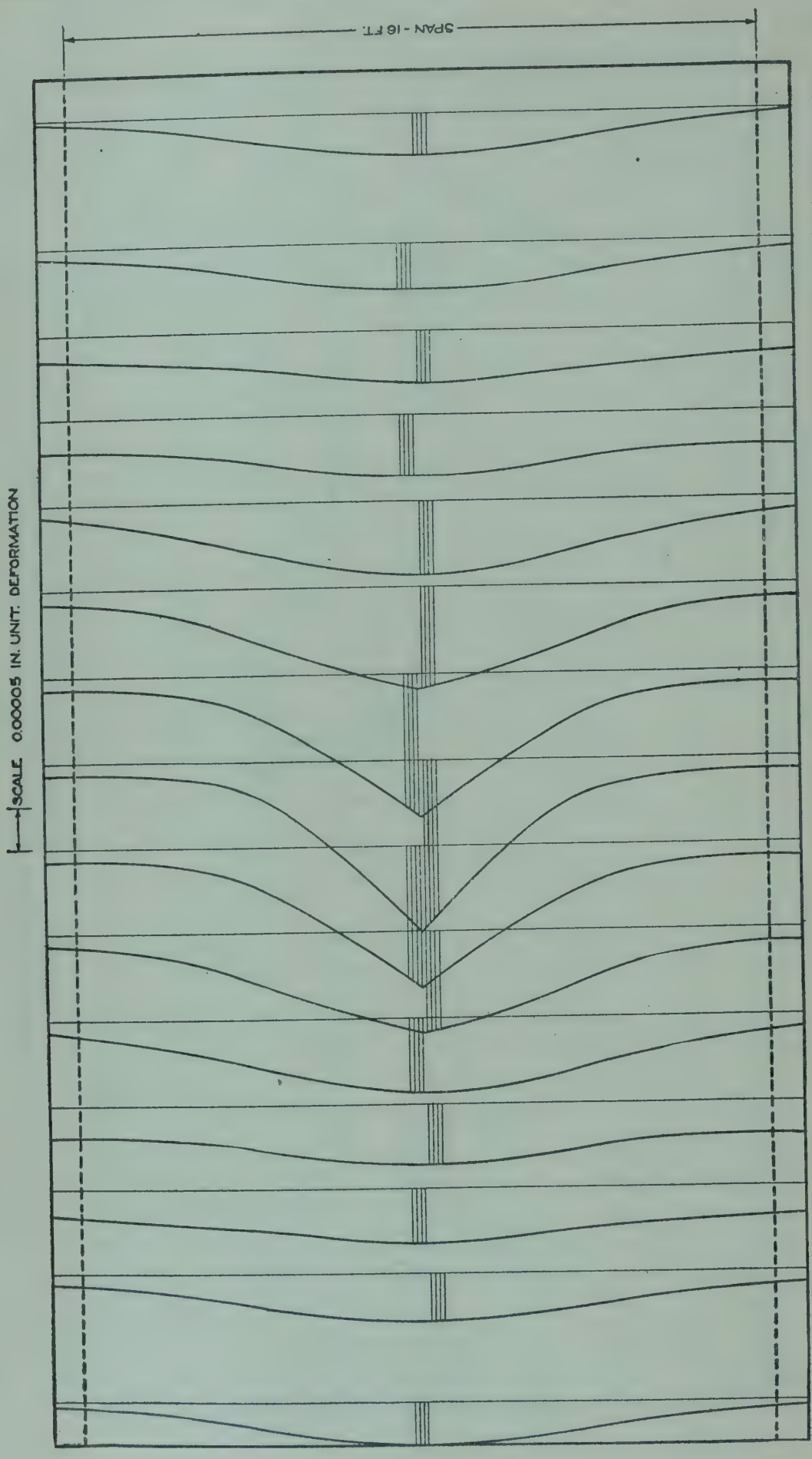



FIG. 21.—Concrete deformation curves for a 10,000-pound concentrated center load on slab 934. Variation in deformations plotted perpendicular to supports.

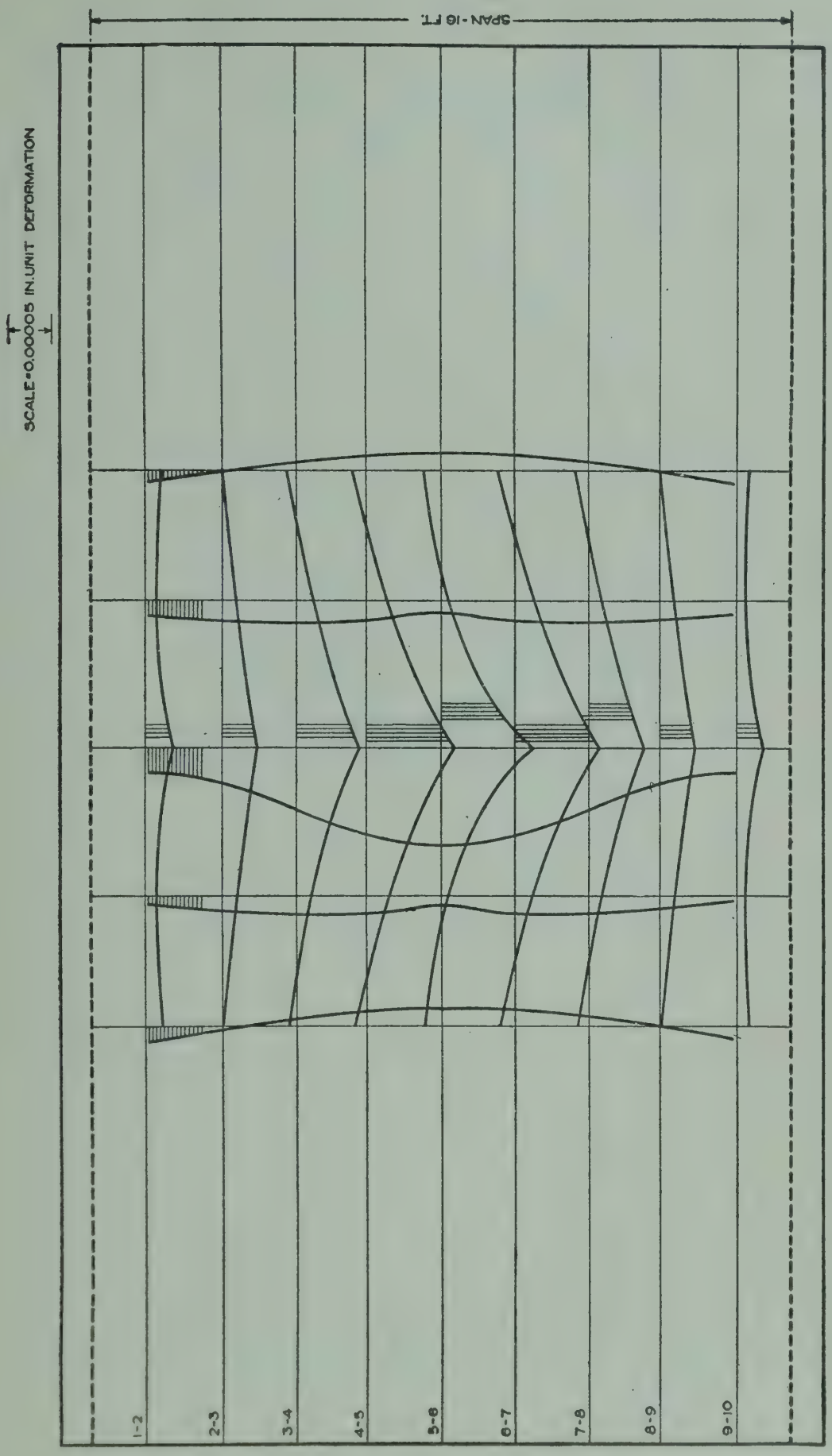



FIG. 22.—Concrete deformation curves for slab 934. Lateral deformations plotted both parallel and perpendicular to supports.

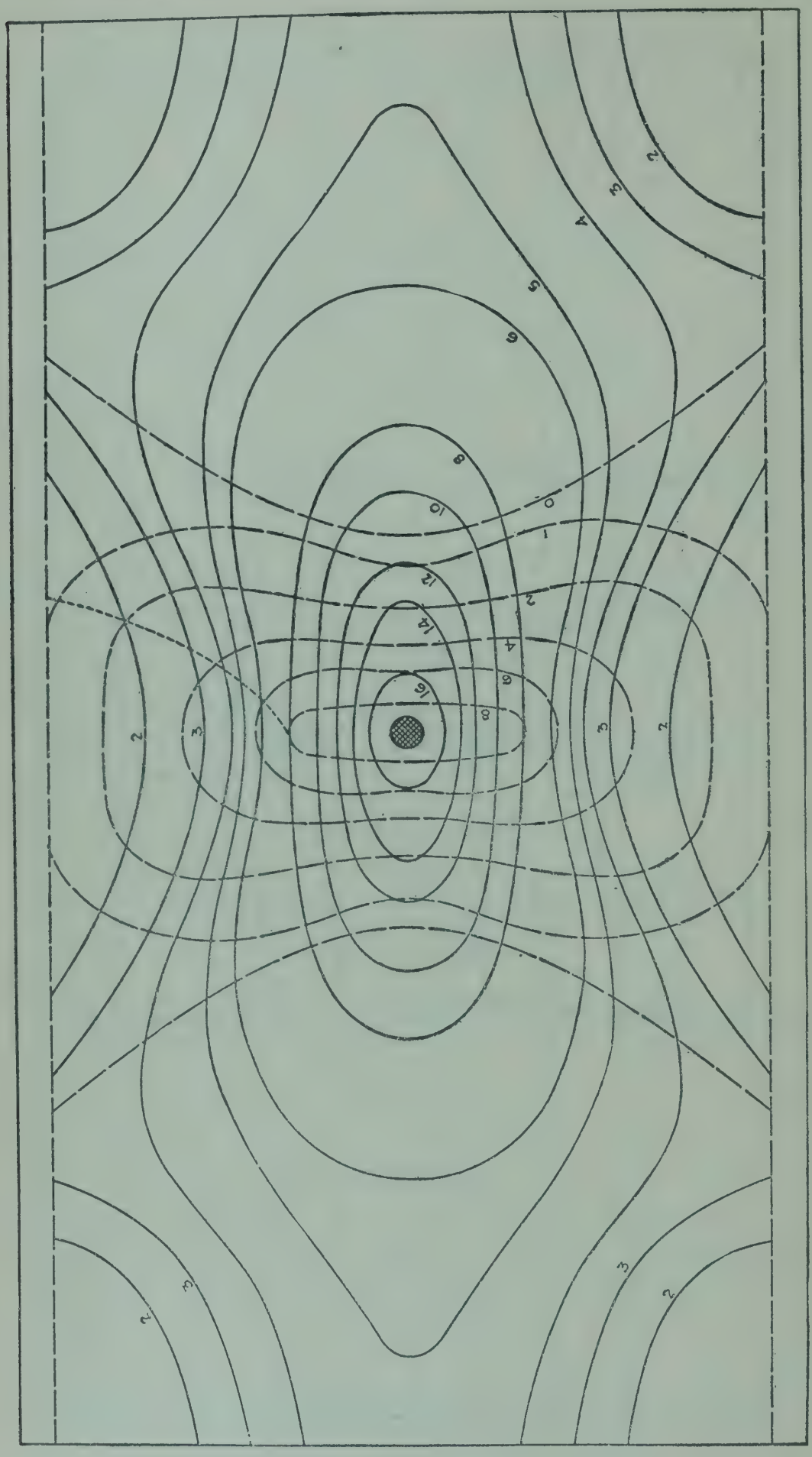



FIG. 23.—Iso-deformation lines for slab 934 under concentrated center load.

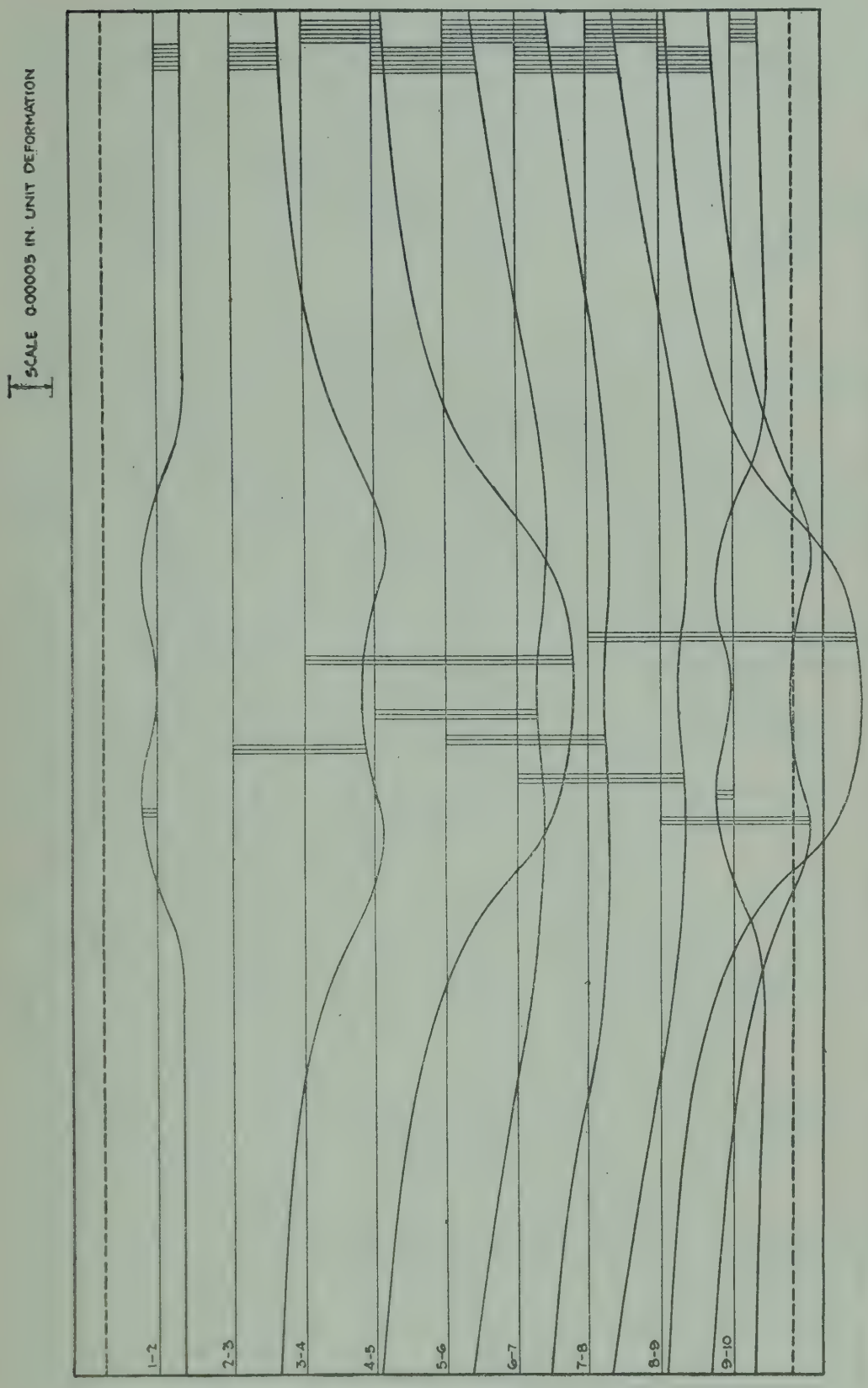



FIG. 24.—Concrete deformation curves for slab 934 under 40,000-pound 4-point loading. Deformations measured perpendicular to supports. Variation of deformations plotted parallel to the supports.

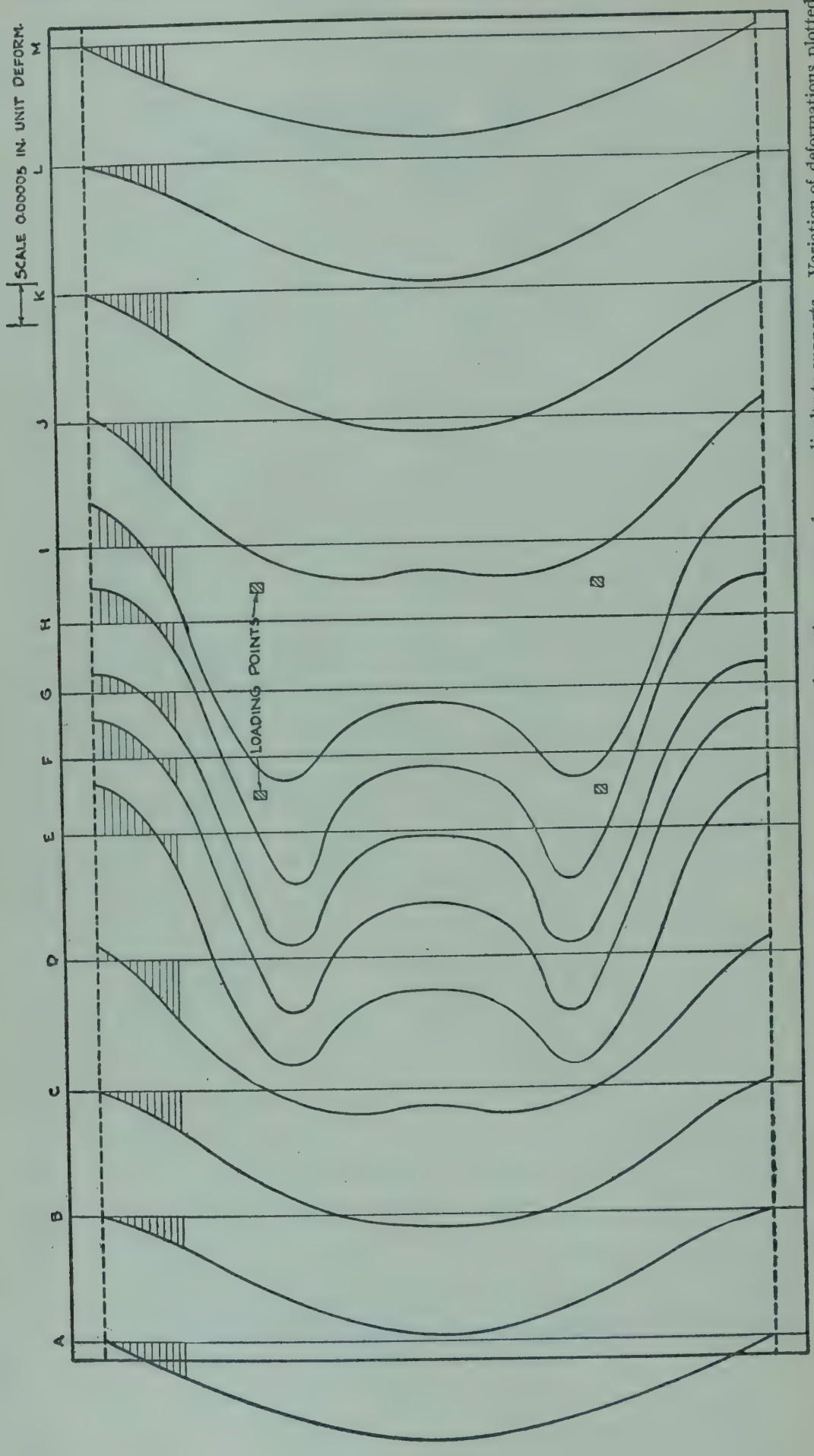



FIG. 25.—Concrete deformation curves for slab 934 under 40,000-pound 4-point loading. Deformations measured perpendicular to supports. Variation of deformations plotted perpendicular to supports.

## CONCLUSION

If figure 27 is referred to, the influence on the effective width of the magnitude of the load and the manner of interpreting the results may be

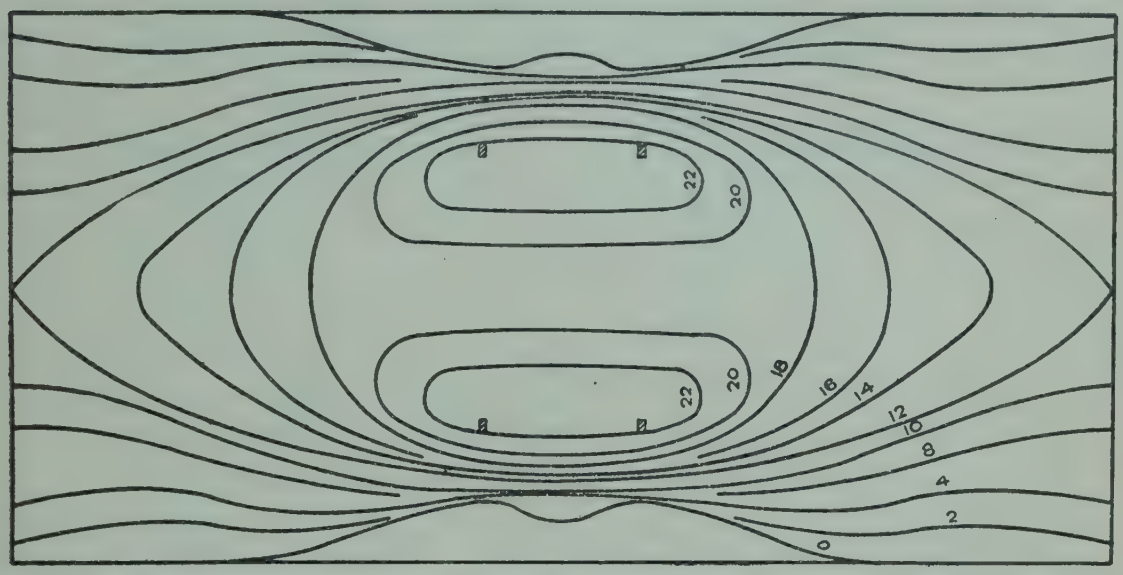



FIG. 26.—Iso-deformation lines for slab 934 under 40,000-pound 4-point loading. Deformations measured perpendicular to supports.

seen. It has been pointed out that the correct method of obtaining deformations is to base all calculations on zero readings taken just before

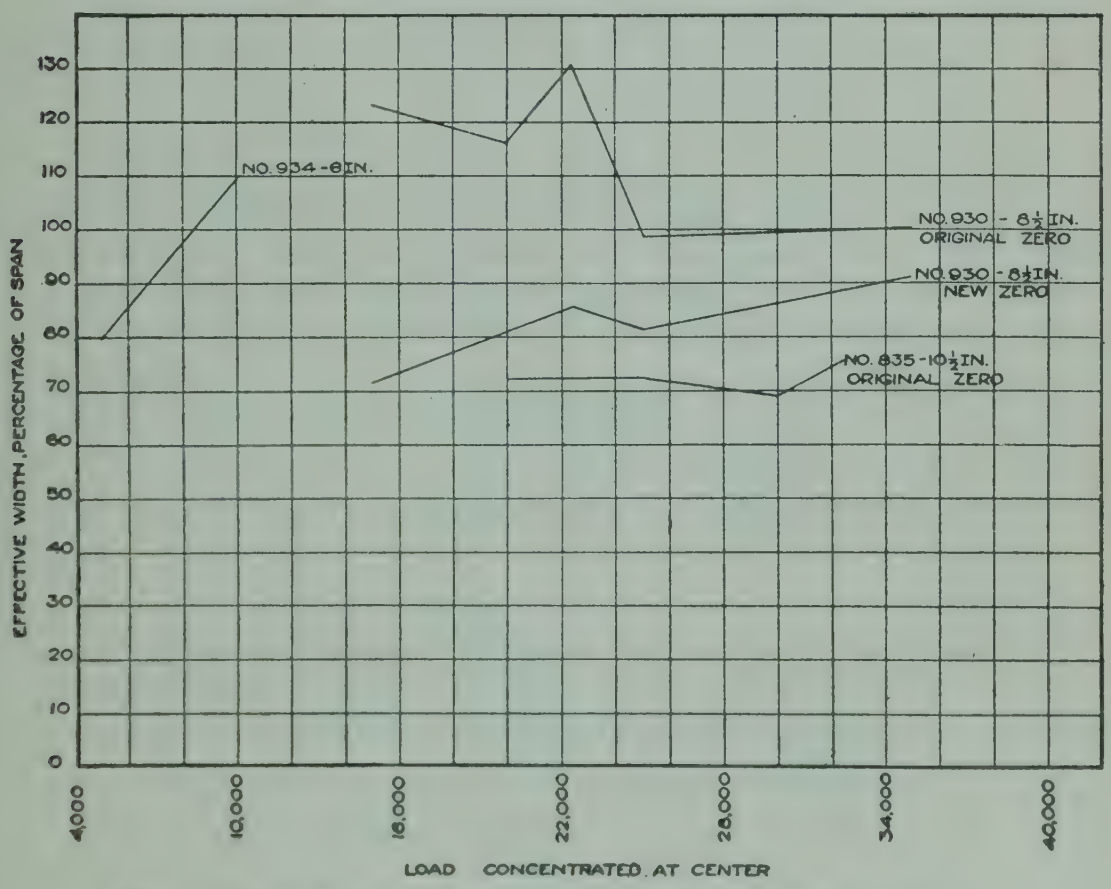



FIG. 27.—Curves showing effective width versus load (concentrated center load).

the load has been applied (designated on the curve as "new zero"). In the case of slab 930, figure 8, note the difference in effective width obtained

depending on the manner of considering the zero readings. The more conservative values are obtained by basing the calculations on the "new zero" readings, as was done in the case of slabs 930 and 934. Note that with an increase in load, the effective width seems to increase slightly. Values for effective width were obtained from the steel deformations, as well as from the concrete deformations, but it was found that the concrete deformations gave the most conservative widths, and these were therefore plotted.

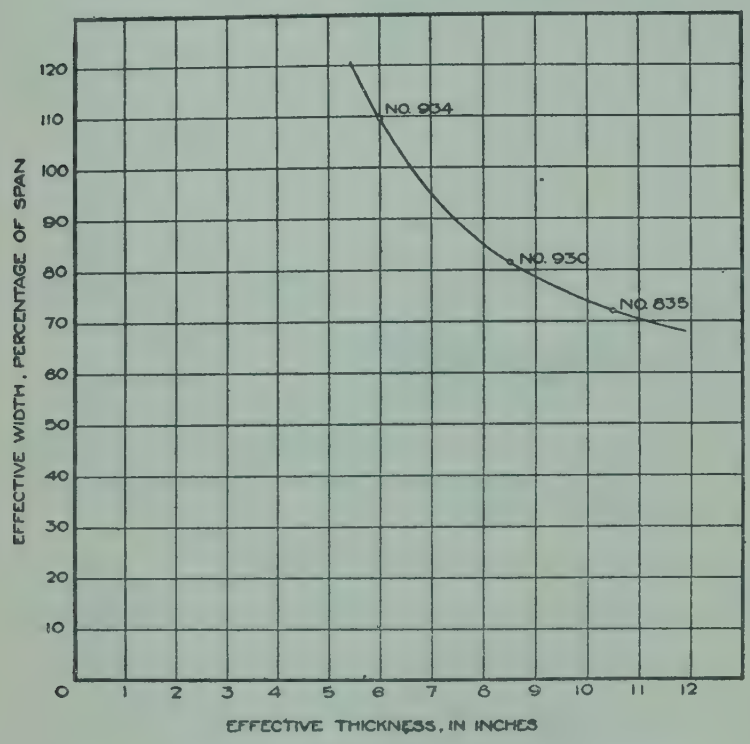



FIG. 28.—Curve showing effective width versus thickness.

In figure 28 the effect of variation in thickness of slab on effective width may be seen. Note that as the thickness increases, the effective width decreases, varying from 109 per cent of the span length for a 6-inch slab to 75 per cent of the span for a 10½-inch slab. The least value for effective width shown by these tests is roughly, then, about 0.7 of the span length. Judging from the curve of variation, it would seem that under extremely heavy loads, requiring very thick slabs, the effective width might be decreased as low, possibly, as 0.6 of the span length. However, 0.7 of the span will always be safe, and in general is a sufficiently conservative figure to use.

TABLE III.—*Effective widths of reinforced-concrete slabs, 16-foot span by 32 feet wide, for center loading*

| Center load. | Slab 835 (10½ inches effective thickness). | Slab 930 (8½ inches effective thickness). | Slab 934 (6 inches effective thickness). |
|---|---|---|---|
| *Pounds.* | | | |
| 15,000 | ..... | 11.4 feet=71.6 per cent of span. | 12.7 feet=79.5 per cent of span. |
| 20,000 | 11.6 feet=72.3 per cent of span. | 13.0 feet=81.2 per cent of span. | 17.5 feet=109.3 per cent of span. |
| 25,000 | 11.5 feet=71.9 per cent of span. | 12.9 feet=81.1 per cent of span. | ..... |
| 32,500 | 12.1 feet=75.7 per cent of span. | ..... | ..... |
| 35,000 | ..... | 14.5 feet=90.7 per cent of span. | ..... |
| Safe load | 12.1 feet=75.7 per cent of span. | 12.9 feet=81.1 per cent of span. | 17.5 feet=109.3 per cent of span. |

## APPLICATION OF RECTANGULAR-BEAM THEORY TO DESIGN OF SLABS UNDER CONCENTRATED LOADS

The usual rectangular-beam design formulas may be applied to the design of slabs by merely substituting for $b$ its value as determined by these investigations, $b=0.7L$. The corresponding formulas then become—

| | FOR RECTANGULAR BEAMS | FOR SLABS UNDER CENTRAL CONCENTRATED LOADS |
|---|---|---|
| (1) | $M_c=\frac{1}{2}f_c kjbd^2$ | $M_c=\frac{1}{2}f_c kj\frac{7}{10}Ld^2$ |
| (2) | $M_s=pf_s jbd^2$ | $M_s=pf_s j\frac{7}{10}Ld^2$ |
| (3) | $p=\frac{a_s}{bd}$ | $p=\frac{10a_s}{7Ld}$ |
| (4) | $p=\frac{1/2}{\frac{f_s}{f_c}\left(\frac{f_s}{nf_c}+1\right)}$ | $p=\frac{1/2}{\frac{f_s}{f_c}\left(\frac{f_s}{nf_c}+1\right)}$ |
| (5) | $k=\sqrt{2pn+(pn)^2}-pn$ | $k=\sqrt{2pn+(pn)^2}-pn$ |

It is interesting to note that in substituting for $M_c$ and $M_s$ in formulas 1 and 2 their value $\frac{PL}{4}$, the $L$'s cancel, showing that the safe load-carrying capacity of the slab is independent of the span; thus—

1 becomes $\frac{PL}{4}=\frac{1}{2}f_c kj\frac{7}{10}Ld^2$ or $P=\frac{7}{5}f_c kjd^2$

2 becomes $\frac{PL}{4}=pf_s j\frac{7}{10}Ld^2$ or $P=p\frac{14}{5}f_s jd^2$

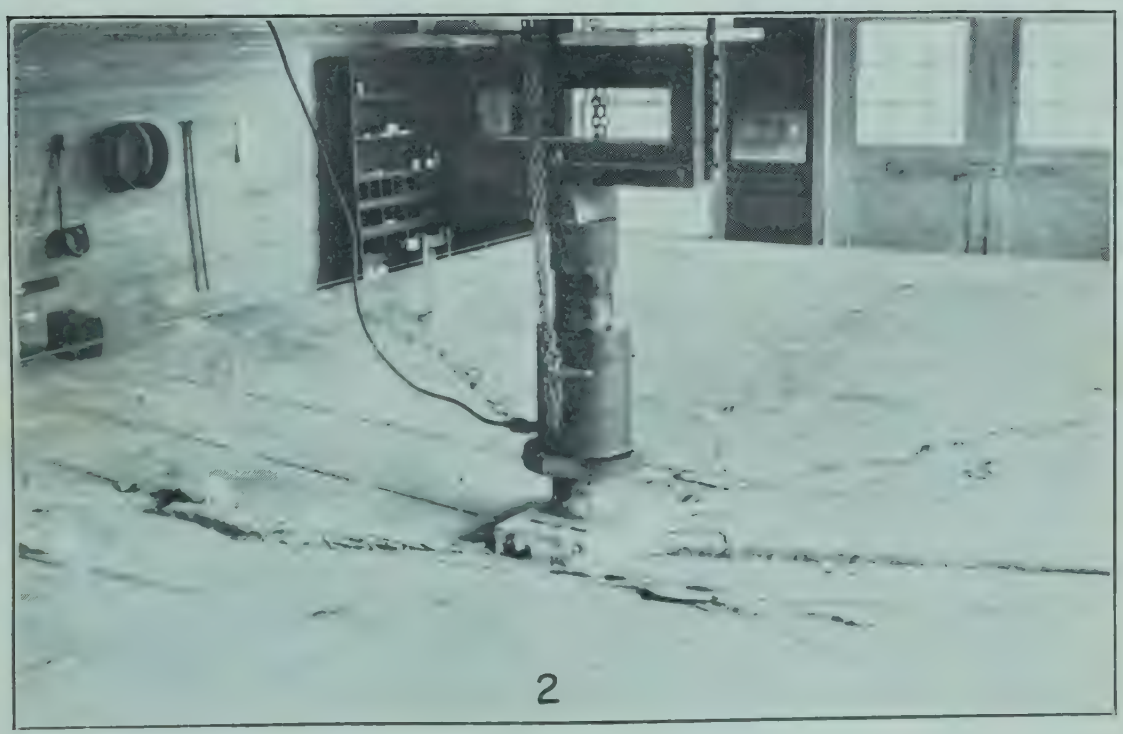
The above investigations were made on slabs the width of which was twice the span length, so that the stress at the extreme edges was very small. The conclusions must therefore be applied to such cases only. When the ratio of width of slab to span length is less than 2, these conclusions may or may not apply, and additional investigations are now being made to determine the proper value of effective width to use under such conditions.

## PLATE XXVI

Fig. 1.—Load-applying and load-measuring apparatus for testing reinforced-concrete slabs, showing set-up for 4-point loading.

Fig. 2.—Load-measuring apparatus and hydraulic jack for testing reinforced-concrete slabs.

1



2

# OCCURRENCE OF STERILE SPIKELETS IN WHEAT

By A. E. GRANTHAM, *Agronomist, Delaware Agricultural Experiment Station*, and FRAZIER GROFF, *Student, Delaware College*

## INTRODUCTION

The average spike of wheat (*Triticum* spp.) contains from 15 to 20 spikelets, each of which under favorable conditions is capable of producing two or more kernels. Ordinarily, however, the lower two or three spikelets on the spike do not develop. The only indications of their absence are the joints or nodes of the rachis which are thus exposed (Pl. XXVII). Hunt states that often in the cultivated varieties and always in the wild species the lower one to four are sterile. In this paper the term "sterile spikelet" is used to designate those spikelets at the base of the spike which for some reason fail to develop and produce seed. No account was taken of the sterile florets which might occasionally occur within the spikelet. The absent spikelets, as shown by the naked rachis, were the only ones estimated as sterile.

## MATERIAL AND METHODS

During the summer of 1915 the writer had the opportunity of making a detailed study of the occurrence of sterile spikelets in a large number of varieties of wheat under test by the Department of Agronomy at the Delaware Agricultural Experiment Station. These varieties and strains of wheat, 188 in number, had been sown the previous autumn by two methods: First, by a grain drill as under ordinary field conditions, at the rate of 7 pecks per acre; second, by the centgener or hill method, leaving the individual plants 6 inches apart each way. By the former method the plants were very close in the rows, which were 8 inches apart. This gave an opportunity to determine to what degree the closeness of the plants or rate of seeding influenced the frequency of sterile spikelets.

The data for each variety were secured in the following manner: The total number of fertile and sterile spikelets were counted on 25 representative spikes of each variety. The means of the fertile spikelets and the sterile spikelets were taken separately and the percentage of sterile spikelets was determined for each variety of wheat. Where the varieties were planted in hills 6 inches apart each way, five plants of five culms each constituted the 25 spikes, the spikelets of which were counted. In this manner the actual number of sterile spikelets and the percentage of the total number of spikelets were determined for the 188 varieties and strains under the two methods of planting.

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## EFFECT OF RATE OF SEEDING ON STERILITY OF SPIKELETS

It was found (see Table I) that the actual number of sterile spikelets per spike (average of 25 spikes) ranged from 1.84, the lowest, to 5.52, the highest, for varieties in drills; and in hills, from 0.28 sterile spikelets, the lowest, to 3.76, the highest. The percentage of sterile spikelets per average spike in drill rows ranged from 11.5 per cent, the lowest, to 36 per cent, the highest. In hills the percentage of sterile spikelets among the varieties ranged from 1.5 per cent to 23.5 per cent. The mean number of sterile spikelets for all varieties in drill rows was 3.47; in hills, 1.73. The mean percentage of sterile spikelets for all varieties in drills was 21.8 per cent; in hills, 10 per cent.

The data indicate that the spacing of the wheat plants has a direct bearing on the number of sterile spikelets. Wheat planted in hills has more space in which to develop and invariably sends up a greater number of tillers than wheat sown in drills. It has also been observed that the period of maturation is prolonged where the wheat plant has more space. Under these conditions the vegetative activity of the plant is more pronounced, as shown by an increased number of culms, broader leaves, and heavier straw.

TABLE I.—*Number and percentage of sterile spikelets on 25 spikes of bearded and smooth varieties of wheat in 1915*

| Variety. | Bearded or smooth. | Total number of spikelets. | | Number of sterile spikelets. | | Percentage of sterile spikelets. | |
|---|---|---|---|---|---|---|---|
| | | Drill. | Hill. | Drill. | Hill. | Drill. | Hill. |
| Acme | B | 15.36 | 15.80 | 3.56 | 3.72 | 23.69 | 23.54 |
| Acme Bred (Maryland) | B | 14.92 | 15.08 | 2.96 | 2.84 | 19.84 | 18.83 |
| Acme Improved (Maryland) | B | 13.12 | 15.68 | 3.04 | 2.80 | 23.17 | 17.83 |
| Ahrens (Indiana) | S | 16.92 | 17.92 | 2.36 | 1.56 | 13.94 | 8.70 |
| American Banner | S | 16.04 | 16.92 | 2.76 | 2.20 | 17.20 | 13.53 |
| American Bronze | S | 17.88 | 19.96 | 3.72 | 2.88 | 20.80 | 14.48 |
| Babcock (Michigan 07664) | B | 14.64 | 14.72 | 3.60 | 2.96 | 24.59 | 20.10 |
| Bearded Purple Straw | B | 15.48 | 15.04 | 3.44 | 2.12 | 22.22 | 14.09 |
| Bearded Winter (Michigan 9850) | B | 16.28 | 16.28 | 3.76 | 2.56 | 23.09 | 15.72 |
| Bearded Winter Fife | B | 18.28 | 17.48 | 3.80 | 2.48 | 20.79 | 14.18 |
| Beechwood Hybrid | S | 15.50 | 16.16 | 2.48 | 1.68 | 16.00 | 10.39 |
| Beloglina | B | 15.78 | 13.52 | 2.84 | 1.88 | 17.99 | 13.90 |
| Berkeley | B | 16.12 | 16.20 | 3.76 | 2.12 | 23.32 | 13.09 |
| Berkeley Awnless | S | 14.12 | 17.08 | 2.00 | 2.32 | 14.16 | 13.58 |
| Blue Stem | S | 15.44 | 15.44 | 2.84 | 1.24 | 18.38 | 8.03 |
| Broughton | S | 15.20 | 15.28 | 2.68 | 1.64 | 17.63 | 10.73 |
| Buda Dawson (Michigan 310717) | B | 14.36 | 14.80 | 3.72 | 1.80 | 25.90 | 12.16 |
| Buda Pest | B | 15.48 | 14.48 | 3.04 | 1.48 | 19.63 | 10.22 |
| Canadian Hybrid | S | 17.40 | 15.80 | 4.12 | 1.20 | 23.67 | 7.59 |
| Century | S | 15.84 | 16.24 | 3.00 | 1.12 | 18.93 | 6.89 |
| China | S | 17.04 | 15.68 | 3.48 | 2.08 | 20.42 | 13.26 |
| Clawsons Longberry | S | 17.64 | 18.24 | 3.40 | 2.00 | 19.27 | 10.96 |
| Cooks Brookmont | B | 13.36 | 15.16 | 3.24 | 2.00 | 24.25 | 13.19 |
| Councilman | S | 15.32 | 15.76 | 2.64 | 1.36 | 17.23 | 8.26 |
| Craigs Favorite | S | 15.48 | 17.44 | 3.56 | 1.76 | 22.99 | 10.09 |
| Currells Prolific | S | 15.52 | 16.32 | 3.48 | 1.12 | 22.39 | 6.86 |
| Crimean | S | 14.64 | 17.28 | 3.48 | 2.04 | 23.77 | 11.80 |

TABLE I.—*Number and percentage of sterile spikelets on 25 spikes of bearded and smooth varieties of wheat in 1915*—Continued

| Variety. | Bearded or smooth. | Total number of spikelets. | | Number of sterile spikelets. | | Percentage of sterile spikelets. | |
|---|---|---|---|---|---|---|---|
| | | Drill. | Hill. | Drill. | Hill. | Drill. | Hill. |
| Dawsons Golden Chaff | S | 16.36 | 17.32 | 3.00 | 2.56 | 18.33 | 14.77 |
| Defiance | B | 13.60 | 13.76 | 3.24 | 1.60 | 23.82 | 11.62 |
| Diamond Grit | B | 18.76 | 18.76 | 5.28 | 3.52 | 28.14 | 18.76 |
| Dietz | B | 15.28 | 15.44 | 3.24 | 1.68 | 21.20 | 10.87 |
| Dietz Longberry | B | 15.04 | 16.55 | 2.86 | 2.16 | 19.01 | 13.19 |
| Doub | B | 15.00 | 13.52 | 3.12 | 1.64 | 20.80 | 12.13 |
| Dunlap | B | 15.24 | 15.00 | 3.92 | 1.84 | 25.72 | 12.26 |
| Early Harvest | S | 15.60 | 15.08 | 2.60 | .84 | 16.66 | 5.57 |
| Early Red Chief | S | 16.24 | 17.56 | 2.64 | .40 | 16.25 | 2.27 |
| Early Red Clawson | S | 16.76 | 16.92 | 3.56 | 1.16 | 21.24 | 6.85 |
| Early Windsor | S | 16.56 | 17.80 | 3.48 | 1.28 | 21.02 | 7.19 |
| Eclipse | B | 17.24 | 19.00 | 3.92 | 2.40 | 22.73 | 12.62 |
| Egyptian Amber | B | 17.64 | 17.08 | 4.72 | 2.80 | 26.75 | 11.63 |
| Enterprise | S | 15.36 | 16.44 | 3.40 | 1.44 | 22.13 | 8.75 |
| European Century | B | 16.76 | 18.48 | 3.32 | 2.08 | 19.80 | 11.25 |
| Farmers Trust | B | 18.08 | 17.40 | 4.48 | 2.24 | 24.77 | 12.87 |
| Fish Head | B | 15.80 | 18.24 | 3.00 | 2.32 | 18.98 | 12.71 |
| Fulcaster | B | 15.56 | 15.28 | 2.96 | 2.12 | 19.02 | 13.87 |
| Four Row Fultz | S | 16.56 | 17.76 | 2.40 | .48 | 14.49 | 2.76 |
| Jersey Fultz | S | 14.84 | 15.64 | 2.72 | .60 | 18.32 | 3.84 |
| Fultz | S | 16.32 | 17.60 | 2.36 | .56 | 14.46 | 3.18 |
| Fultz Mediterranean | S | 16.40 | 16.92 | 1.96 | .28 | 11.95 | 1.59 |
| Genessee Giant | B | 18.04 | 19.44 | 4.20 | 1.28 | 23.28 | 6.58 |
| Giant Square Head | B | 17.40 | 18.36 | 4.28 | .64 | 24.59 | 3.48 |
| Goens | B | 15.36 | 15.00 | 3.24 | 1.04 | 21.09 | 6.92 |
| Goens Awnless | S | 15.44 | 15.32 | 2.48 | .76 | 16.06 | 4.96 |
| Gill | S | 15.44 | 15.12 | 2.52 | .36 | 16.32 | 2.38 |
| Glace | S | 19.00 | 19.80 | 4.68 | 2.08 | 24.63 | 10.50 |
| Gold Coin | S | 17.24 | 17.36 | 3.60 | 1.44 | 20.88 | 8.29 |
| Golden Bronze | S | 16.24 | 18.32 | 3.20 | 2.04 | 19.70 | 11.13 |
| Greening (Michigan 126) | S | 16.04 | 17.92 | 3.52 | 1.80 | 21.94 | 10.04 |
| Gypsy | B | 17.76 | 18.84 | 4.16 | 2.24 | 23.42 | 11.88 |
| Hedges Prolific | S | 15.32 | 16.80 | 2.80 | .44 | 18.27 | 2.16 |
| Hercules | B | 15.16 | 17.08 | 3.20 | 1.88 | 21.10 | 11.00 |
| Harvest King | S | 15.72 | 16.20 | 2.64 | .60 | 16.79 | 3.70 |
| Hickman | S | 15.45 | 15.68 | 2.44 | 1.48 | 15.84 | 9.43 |
| Hungarian (Michigan 913802) | B | 14.92 | 17.96 | 3.92 | 1.80 | 26.27 | 10.02 |
| Hybrid Sel. 13 | B | 18.00 | 22.08 | 3.84 | 2.56 | 21.33 | 11.59 |
| Hyde Michigan 6 | S | 16.84 | 19.88 | 3.24 | 2.12 | 19.23 | 10.66 |
| Imperial Amber | B | 15.92 | 18.68 | 4.04 | 2.56 | 25.37 | 13.70 |
| International 6 (Michigan 61) | S | 15.63 | 18.00 | 3.20 | 1.88 | 20.47 | 10.44 |
| Jones Early Red Chaff | S | 15.80 | 17.76 | 3.28 | .92 | 20.75 | 5.18 |
| Jones Longberry | S | 18.32 | 17.88 | 4.28 | .48 | 23.36 | 2.68 |
| Jones Mammoth Amber | B | 18.96 | 22.68 | 5.00 | 2.36 | 26.37 | 10.40 |
| Jones Paris Prize | S | 16.88 | 18.12 | 3.00 | 1.48 | 17.77 | 8.22 |
| Jones Winter Fife | S | 19.60 | 20.20 | 3.08 | 1.04 | 15.71 | 5.14 |
| Kansas Mortgage Lifter | B | 14.64 | 16.20 | 2.60 | 1.44 | 17.76 | 8.88 |
| K. B. 2 | S | 18.44 | 20.12 | 3.84 | 1.68 | 20.82 | 8.34 |
| Kharkov | B | 13.96 | 15.76 | 3.16 | 1.16 | 22.63 | 7.36 |
| Klondike | S | 16.84 | 17.92 | 3.16 | 1.32 | 18.76 | 7.42 |
| Lancaster-Fulcaster | B | 14.32 | 15.24 | 3.16 | 1.44 | 22.06 | 9.44 |
| Lancaster Red | B | 15.84 | 16.36 | 4.12 | 1.52 | 26.01 | 9.29 |
| Lebanon | B | 14.88 | 15.84 | 3.52 | 2.16 | 23.65 | 13.63 |
| Mammoth Red | B | 15.80 | 16.84 | 2.88 | 2.00 | 18.22 | 11.87 |
| Martins Amber | S | 18.24 | 21.00 | 4.08 | 2.32 | 22.36 | 11.04 |
| Malakoff | B | 14.24 | 14.96 | 2.48 | 1.64 | 17.41 | 10.96 |
| Massey | S | 16.88 | 20.04 | 2.44 | 2.24 | 14.45 | 11.12 |

TABLE I.—*Number and percentage of sterile spikelets on 25 spikes of bearded and smooth varieties of wheat in 1915*—Continued

| Variety. | Bearded or smooth. | Total number of spikelets. | | Number of sterile spikelets. | | Percentage of sterile spikelets. | |
|---|---|---|---|---|---|---|---|
| | | Drill. | Hill. | Drill. | Hill. | Drill. | Hill, |
| Mealy | S | 17. 72 | 20. 00 | 2. 84 | 2. 16 | 16. 02 | 10. 80 |
| Mediterranean | B | 15. 20 | 16. 00 | 3. 68 | 1. 96 | 24. 12 | 12. 25 |
| Michigan Amber | S | 15. 92 | 17. 52 | 3. 00 | 1. 64 | 18. 84 | 9. 36 |
| Millers Pride | B | 14. 20 | 16. 84 | 2. 64 | 2. 20 | 18. 59 | 13. 06 |
| Miracle | B | 15. 72 | 16. 84 | 4. 40 | 1. 80 | 27. 98 | 10. 68 |
| Missing Link | B | 17. 76 | 19. 84 | 4. 64 | 2. 48 | 26. 12 | 12. 50 |
| Morse | S | 14. 48 | 16. 08 | 2. 24 | . 56 | 15. 47 | 3. 48 |
| New Amber Longberry | B | 18. 80 | 20. 16 | 4. 84 | 1. 40 | 25. 73 | 6. 99 |
| New Soules | S | 17. 16 | 17. 80 | 3. 58 | 1. 24 | 20. 86 | 6. 96 |
| Nigger | B | 12. 80 | 14. 40 | 2. 68 | 1. 56 | 20. 93 | 10. 83 |
| Nixon | S | 15. 96 | 15. 60 | 3. 50 | . 84 | 21. 92 | 5. 38 |
| Ohio 5507 | S | 17. 28 | 17. 92 | 3. 72 | 1. 04 | 21. 52 | 5. 80 |
| Ontario Wonder | S | 17. 80 | 20. 32 | 4. 20 | 1. 76 | 23. 59 | 8. 66 |
| Orange | S | 15. 08 | 15. 96 | 2. 72 | 1. 68 | 18. 03 | 10. 53 |
| Pesterboden | B | 15. 16 | 15. 96 | 2. 80 | 1. 80 | 18. 46 | 11. 90 |
| Perfection | S | 14. 92 | 16. 44 | 2. 92 | . 60 | 19. 57 | 3. 64 |
| Plymouth Rock | S | 16. 72 | 18. 88 | 3. 52 | 2. 00 | 21. 05 | 10. 59 |
| Poole | S | 15. 64 | 18. 20 | 2. 72 | 2. 28 | 17. 39 | 12. 52 |
| Pride of Genessee | B | 18. 76 | 21. 48 | 5. 32 | 3. 76 | 28. 35 | 17. 50 |
| Prosperity | S | 16. 84 | 19. 56 | 3. 24 | 1. 60 | 19. 23 | 8. 17 |
| Purple Straw | B | 15. 28 | 20. 44 | 2. 72 | 2. 32 | 17. 80 | 11. 35 |
| Red Cross | S | 16. 60 | 20. 32 | 3. 44 | 1. 24 | 20. 72 | 6. 10 |
| Red Hussar | B | 12. 76 | 18. 12 | 2. 88 | . 72 | 22. 57 | 3. 97 |
| Red Rock | B | 13. 32 | 15. 68 | 3. 36 | 1. 92 | 25. 22 | 12. 24 |
| Red Wave | S | 18. 16 | 19. 52 | 4. 24 | 1. 56 | 23. 34 | 7. 83 |
| Reiti | B | 17. 20 | 21. 60 | 4. 44 | 2. 20 | 25. 81 | 10. 18 |
| Reliable | B | 15. 88 | 18. 68 | 3. 84 | 2. 24 | 24. 18 | 11. 99 |
| Rochester Red | S | 15. 96 | 18. 80 | 3. 96 | 1. 68 | 24. 81 | 8. 93 |
| Rocky Mountain | B | 15. 00 | 15. 68 | 3. 96 | 1. 92 | 26. 40 | 12. 24 |
| Royal Red Clawson | S | 14. 96 | 16. 52 | 3. 24 | . 48 | 21. 65 | 2. 90 |
| Rudy | B | 12. 52 | 14. 20 | 2. 72 | 1. 04 | 20. 92 | 7. 32 |
| Rudy Hard | B | 13. 84 | 16. 12 | 2. 92 | 1. 32 | 21. 09 | 8. 18 |
| Ruperts Giant | S | 17. 24 | 19. 80 | 4. 40 | 1. 60 | 25. 51 | 8. 08 |
| Rural New Yorker | S | 16. 92 | 19. 56 | 3. 72 | 1. 44 | 21. 98 | 7. 36 |
| Russian Amber | B | 15. 84 | 19. 52 | 4. 80 | 2. 76 | 30. 30 | 14. 13 |
| Shepherds Perfection | B | 16. 64 | 20. 56 | 4. 56 | 2. 80 | 27. 40 | 13. 61 |
| Silver Sheath | B | 13. 52 | 17. 52 | 3. 68 | 2. 40 | 27. 14 | 13. 69 |
| Silver Wave | B | 17. 32 | 19. 48 | 4. 76 | 2. 88 | 27. 48 | 14. 79 |
| Smiths Rustproof | S | 16. 72 | 20. 68 | 4. 28 | 2. 72 | 25. 59 | 13. 15 |
| Soumans Champion | B | 16. 80 | 19. 28 | 3. 76 | 1. 88 | 22. 38 | 9. 74 |
| Spayde | B | 16. 52 | 19. 76 | 4. 16 | 1. 56 | 25. 18 | 7. 89 |
| St. Louis Grand Prize | S | 18. 00 | 20. 40 | 3. 44 | 1. 24 | 19. 11 | 6. 07 |
| Stone | B | 13. 00 | 16. 96 | 3. 84 | 2. 32 | 29. 53 | 13. 68 |
| Swamp | B | 14. 88 | 17. 88 | 3. 68 | . 84 | 24. 73 | 4. 69 |
| Theiss | B | 13. 28 | 17. 52 | 3. 84 | 1. 60 | 28. 91 | 9. 70 |
| Turkey Red | B | 14. 44 | 16. 60 | 2. 76 | 1. 72 | 19. 11 | 10. 37 |
| Turkish Amber | B | 15. 48 | 16. 88 | 3. 28 | 1. 24 | 21. 18 | 7. 34 |
| Velvet Chaff | B | 16. 80 | 18. 04 | 4. 04 | 1. 88 | 24. 04 | 10. 42 |
| Valley | ..... | 16. 68 | 17. 76 | 4. 60 | 2. 32 | 27. 57 | 13. 06 |
| Wayside Wonder | B | 14. 32 | 17. 08 | 3. 92 | 1. 68 | 27. 37 | 9. 83 |
| Whedling | S | 13. 80 | 15. 60 | 2. 40 | . 92 | 17. 39 | 5. 89 |
| White Eldorado | S | 15. 96 | 18. 36 | 3. 48 | 1. 24 | 21. 80 | 6. 75 |
| Wyandotte Red | S | 14. 64 | 16. 96 | 2. 80 | 1. 16 | 19. 12 | 6. 82 |
| Tennessee 3608 | B | 18. 12 | 22. 48 | 5. 52 | 3. 12 | 30. 46 | 13. 87 |
| Tennessee 3609 | B | 17. 60 | 19. 68 | 4. 40 | 2. 20 | 25. 00 | 11. 17 |
| Tennessee 3611 | B | 15. 16 | 20. 16 | 4. 32 | 2. 76 | 28. 49 | 13. 69 |
| Tennessee 3614 | B | 15. 28 | 18. 40 | 3. 80 | 2. 40 | 24. 86 | 13. 04 |

TABLE I.—*Number and percentage of sterile spikelets on 25 spikes of bearded and smooth varieties of wheat in 1915*—Continued

| Variety. | Bearded or smooth. | Total number of spikelets. | | Number of sterile spikelets. | | Percentage of sterile spikelets. | |
|---|---|---|---|---|---|---|---|
| | | Drill. | Hill. | Drill. | Hill. | Drill. | Hill. |
| Tennessee 3617 | B | 17.16 | 20.44 | 4.68 | 2.36 | 27.27 | 11.54 |
| Tennessee 3277 | B | 18.36 | 21.60 | 5.40 | 2.64 | 29.41 | 12.22 |
| U. S. 2980 | B | 14.84 | 17.08 | 2.92 | 2.00 | 19.67 | 11.70 |
| U. S. 3608 | B | 17.92 | 21.24 | 4.84 | 2.72 | 27.06 | 12.80 |
| U. S. 3609 | B | 17.12 | 19.68 | 4.36 | 2.20 | 25.46 | 11.17 |
| U. S. 3610 | S | 18.16 | 21.56 | 3.52 | 1.68 | 19.38 | 7.79 |
| U. S. 3612 | B | 17.52 | 21.36 | 4.48 | 2.52 | 25.57 | 11.79 |
| U. S. 3613 | B | 15.00 | 20.40 | 3.92 | 2.56 | 26.13 | 12.54 |
| U. S. 3614 | B | 16.40 | 19.20 | 3.40 | 1.80 | 20.73 | 9.36 |
| Abundance | S | 16.44 | 17.76 | 2.60 | 2.00 | 15.81 | 11.26 |
| Auburn Red | B | 16.08 | 16.88 | 3.40 | 2.32 | 21.15 | 13.74 |
| Australian Red | B | 14.52 | 15.84 | 3.24 | 2.12 | 22.31 | 13.38 |
| Banat | B | 13.72 | 15.52 | 3.48 | 2.64 | 25.36 | 17.01 |
| Bulgarian | B | 14.88 | 16.68 | 4.16 | 2.04 | 27.95 | 12.23 |
| California Red | S | 14.96 | 15.76 | 2.88 | .32 | 19.25 | 2.03 |
| Davidson | S | 15.95 | 17.44 | 1.84 | .80 | 11.52 | 4.58 |
| Deitz Amber | B | 14.52 | 15.88 | 3.80 | 1.24 | 26.17 | 7.80 |
| Deitz Mediterranean | B | 14.44 | 15.64 | 3.50 | 1.32 | 24.23 | 8.43 |
| Early Pearl | S | 13.04 | 13.92 | 2.56 | .72 | 19.64 | 5.17 |
| Early Ripe | S | 15.08 | 16.08 | 2.80 | .68 | 18.56 | 4.22 |
| Economy | S | 14.48 | 15.36 | 2.72 | 1.16 | 18.78 | 7.55 |
| Egyptian | B | 14.08 | 17.20 | 5.08 | 1.80 | 36.07 | 10.46 |
| Farmers Friend | B | 13.00 | 14.40 | 2.96 | 1.68 | 22.76 | 11.69 |
| Ghirka Winter | B | 15.76 | 18.56 | 3.92 | 2.12 | 28.88 | 11.36 |
| Goings | B | 15.16 | 14.80 | 3.00 | 1.04 | 19.78 | 7.02 |
| Grand Prize | S | 17.76 | 19.72 | 3.36 | 2.00 | 18.91 | 10.14 |
| Invincible | S | 17.92 | 20.60 | 4.36 | 2.32 | 24.33 | 11.26 |
| Jones Red Wave | S | 18.20 | 20.21 | 4.24 | 1.64 | 23.29 | 8.15 |
| Kentucky Bluestem | S | 14.84 | 17.28 | 3.12 | 1.40 | 21.02 | 8.10 |
| Lancaster | B | 14.36 | 15.16 | 3.48 | 1.96 | 24.23 | 12.92 |
| Lehigh | B | 13.96 | 17.16 | 3.52 | 2.88 | 25.21 | 16.78 |
| Petigree Giant | B | 18.24 | 19.40 | 3.80 | 1.40 | 20.83 | 7.21 |
| Red May | S | 14.32 | 16.84 | 2.64 | .36 | 18.43 | 2.13 |
| Reiti | B | 14.72 | 17.88 | 3.44 | 1.68 | 25.14 | 9.39 |
| Sibleys New Golden | B | 14.44 | 17.84 | 3.56 | 1.52 | 24.65 | 8.54 |
| Texas Red | B | 13.04 | 17.28 | 3.80 | 1.76 | 29.14 | 16.18 |
| Treadwell | B | 15.24 | 18.00 | 4.60 | 1.72 | 30.18 | 9.55 |
| Tuscan Island | B | 15.32 | 16.72 | 3.92 | 2.00 | 25.58 | 11.96 |
| Ulta | B | 13.32 | 16.04 | 3.80 | 1.24 | 28.52 | 7.73 |
| Winter Chief | S | 15.44 | 17.56 | 2.40 | .44 | 15.54 | 2.50 |
| Winter King | B | 14.16 | 15.04 | 3.24 | 1.28 | 22.88 | 8.51 |
| Wisconsin 13 | B | 13.12 | 16.36 | 3.76 | 1.24 | 28.65 | 7.58 |
| Leaps Prolific | S | 17.06 | 18.88 | 2.28 | .68 | 13.36 | 3.60 |
| Average | ..... | 15.85 | 17.13 | 3.47 | 1.73 | 21.88 | 10.09 |

Of the 188 varieties and strains of wheat under observation, 108 were beardless and 80 bearded. To determine whether the presence or absence of awns as a morphological character was in any way correlated with the occurrence of sterile spikelets, the varieties were tabulated so as to show the distribution of bearded and of beardless varieties with reference to the percentage of spikelets (see Table II). The data in this case were taken from the varieties sown in drills.

TABLE II.—*Arrangement of bearded and beardless varieties of wheat with reference to the percentage of sterile spikelets*

| Percentage of barren spikelets. | Total number of varieties. | Number of beardless varieties. | Number of bearded varieties. | Percentage of each class to total number of— | |
|---|---|---|---|---|---|
| | | | | Beardless varieties. | Bearded varieties. |
| 11 to 15 | 8 | 8 | 0 | 10.0 | 0 |
| 15 to 17 | 12 | 12 | 0 | 15.0 | 0 |
| 17 to 19 | 27 | 19 | 8 | 23.7 | 7.4 |
| 19 to 21 | 32 | 18 | 14 | 22.5 | 12.9 |
| 21 to 23 | 30 | 14 | 16 | 17.5 | 14.8 |
| 23 to 25 | 31 | 7 | 24 | 8.7 | 22.2 |
| 25 to 27 | 25 | 2 | 23 | 2.5 | 21.2 |
| 27 to 29 | 17 | 0 | 17 | 0 | 15.7 |
| 29 to 31 | 5 | 0 | 5 | 0 | 5.5 |
| Total | 188 | 80 | 108 | 100.0 | 100.0 |

Table II shows that the bearded varieties as a class have a higher percentage of sterile spikelets than the beardless wheats. There are 20 of the 80 varieties of beardless wheat which have more than 15 per cent of sterile spikelets, while not a single variety of bearded wheat has less than 17 per cent of sterile spikelets. Of the 108 bearded varieties 45 have not less than 25 per cent of sterile spikelets. Only two of the 80 beardless varieties have 25 per cent of sterile spikelets. The average percentage of sterile spikelets for all the beardless varieties is 17.8; for the bearded, 24.1; a difference of 6.1 per cent in favor of the beardless varieties. The individual variety having the lowest percentage, 11.5, was beardless, while the variety having the highest percentage of sterile spikelets, 36.7, was bearded. All of the varieties which are mentioned above were sown under like conditions of soil preparation and fertilization and planted at the same time.

## EFFECT OF TIME OF SEEDING ON STERILITY

The next step was to determine the effect of time of seeding and of soil treatment on the frequency of sterile spikelets. As it happened, an experiment was already under way on different dates of sowing wheat, including two varieties, one bearded and the other beardless, on both fertilized and unfertilized soil. These plants were in hills 6 inches apart each way. In the manner followed above, the total number of spikelets and that of sterile spikelets per spike were combined, and the average was determined for the two varieties under different dates of planting on both treated and untreated soil (Table III).

TABLE III.—*Effect of date of planting on the number of sterile spikelets in 25 spikes of two varieties of wheat on fertilized and on unfertilized soil*

RED WAVE (BEARDLESS)

| Date of planting. | Total number of spikelets. | | Number of sterile spikelets. | | Percentage of sterile spikelets. | |
|---|---|---|---|---|---|---|
| | Fertilizer. | No fertilizer. | Fertilizer. | No fertilizer. | Fertilizer. | No fertilizer. |
| Sept. 17 | 21.4 | 17.7 | 2.8 | 2.1 | 13.4 | 12.1 |
| 24 | 20.5 | 18.4 | 2.2 | 1.8 | 11.1 | 9.7 |
| Oct. 1 | 20.0 | 20.3 | 2.2 | 2.2 | 11.1 | 10.8 |
| 8 | 21.5 | 18.8 | 2.1 | 1.5 | 10.1 | 8.4 |
| 15 | 21.3 | 19.9 | 2.2 | 1.6 | 10.6 | 8.4 |
| 22 | 19.7 | 20.9 | 1.0 | 1.2 | 5.4 | 5.7 |
| Average | 20.7 | 19.3 | 2.1 | 1.7 | 10.3 | 9.3 |

MIRACLE (BEARDED)

| | | | | | | |
|---|---|---|---|---|---|---|
| Sept. 17 | 16.7 | 15.0 | 2.3 | 1.5 | 13.8 | 10.4 |
| 24 | 16.2 | 14.9 | 2.6 | 1.2 | 16.4 | 8.5 |
| Oct. 1 | 18.9 | 15.5 | 2.6 | 1.8 | 14.1 | 11.5 |
| 8 | 16.0 | 15.4 | 3.1 | 1.8 | 19.7 | 12.1 |
| 15 | 15.7 | 16.8 | 1.6 | 1.8 | 10.1 | 10.9 |
| 22 | 16.1 | 15.4 | .9 | .4 | 5.7 | 2.8 |
| Average | 16.6 | 15.5 | 2.2 | 1.4 | 13.3 | 9.4 |

Table III shows that the number of sterile spikelets per spike varies considerably from the earliest seeding, September 17, to the latest, October 22, but in no regular manner. The latest seeding in every case shows the smallest number of sterile spikelets. This holds true for both varieties and under both soil conditions. If the average is taken of the number of sterile spikelets under the six different dates of seeding, it is found that there are more sterile spikelets where fertilizer was used than where no application was made. This also holds true for both varieties. Expressed as a percentage, the average of sterile spikelets for the different rates of seeding with the beardless variety is 10.3 per cent where fertilizer was used and 9.3 per cent on untreated soil. That of the bearded variety was 13.3 per cent of sterile spikelets as an average for the different dates of seeding on treated soil and 9.4 per cent on the untreated. It will be noted that the latest seeding of each variety has as many spikelets as the earliest, and that there are more than twice as many sterile spikelets in the latter than in the former. This may be partially accounted for by the fact that the later plantings did not have a full stand of plants, thus giving the individual wheat plant more space. This explanation is in accord with results obtained under the different methods of seeding (see Table I)—that is, that fewer sterile spikelets were found in the thinner plantings.

The tillering in the early plantings was nearly 100 per cent greater than in the later plantings. The tillering for each variety on fertilized soil for a given date was 50 per cent greater than where no fertilizer was used. The general effect of the date of seeding seems to indicate a tendency toward a smaller percentage of sterile spikelets in the later seedings. The relation of the number of sterile spikelets to yield does not seem to affect the yield seriously, since the fertilized wheats produced two or three times as much grain per spike as the unfertilized. The difference in yield per spike seems to be due largely to quality (size) of kernel.

TABLE IV.—*Relation of the effect of different fertilizers and combinations of fertilizers to the occurrence of sterile spikelets*

| Treatment. | Dawsons Golden Chaff (smooth). | | | Lehigh (bearded). | | |
|---|---|---|---|---|---|---|
| | Total number of spikelets.[1] | Number of sterile spikelets.[1] | Percentage of sterile spikelets. | Total number of spikelets.[1] | Number of sterile spikelets.[1] | Percentage of sterile spikelets. |
| Nitrogen, phosphorus, and potassium | 16.8 | 1.36 | 8.0 | 17.9 | 1.32 | 7.3 |
| Nitrogen and phosphorus | 18.2 | 1.68 | 9.2 | 18.2 | 2.08 | 11.4 |
| Phosphorus and potassium | 18.2 | 1.92 | 10.2 | 17.2 | 1.80 | 10.4 |
| Nitrogen and potassium | 18.2 | 1.08 | 5.9 | 17.3 | .92 | 5.2 |
| None | 17.0 | 1.05 | 6.1 | 16.7 | 1.01 | 6.0 |
| Nitrogen | 16.9 | .92 | 5.4 | 18.0 | 1.36 | 7.5 |
| Phosphorus | 15.9 | 1.56 | 9.7 | 15.0 | 1.40 | 9.2 |
| Potassium | 17.0 | 1.32 | 7.7 | 16.8 | 1.24 | 7.3 |

[1] Average of 25 spikes.

## EFFECT OF FERTILIZERS ON STERILITY

The effect of different elements of plant food, singly and in combination, on the number of sterile spikelets is seen in Table IV. The wheat was planted by the centgener method, the individual plants being 6 inches apart each way. On each of the plots sufficient fertilizer of each mineral ingredient was supplied to produce a 50-bushel crop of wheat, provided that it were all used. The nitrogen was applied for a 25-bushel crop, it being assumed that the soil carried a fair reserve of this element. The nitrogen was applied in equal parts by weight of nitrate of soda and dried blood; the phosphoric acid was carried as acid phosphate and the potash as muriate of potash. It will be noted that where the fertilizers were applied singly nitrogen gave the lowest percentage—6.4—of sterile spikelets as an average for the two varieties. Potash came next with 7.5 per cent, and phosphoric acid stood highest, with 9.4 per cent of sterile spikelets. Where two elements were used in combination, phosphoric acid and potash led, with an average of

10.4 per cent for the two varieties; phosphoric acid and nitrogen combined gave 10.3 per cent of sterile spikelets, while nitrogen and potash gave 5.4 per cent. Since phosphoric acid gave the highest percentage of sterile spikelets when used alone, it would seem that this element of plant food is largely responsible for the sterile spikelets, as in every combination in which it is used the number of sterile spikelets is greater than where nitrogen and potash are used singly or in combination. The untreated plot gave 6 per cent of sterile spikelets, the lowest for the series except where nitrogen and potash were used in combination, which gave 5.5 per cent. The complete fertilizer gave an average of 7.6 per cent of sterile spikelets. From these data it would seem that there is a tendency for phosphoric acid to produce a larger percentage of sterile spikelets than either potash or nitrogen. However, the fairly high percentage of sterile spikelets in the case of the wheat treated with phosphoric acid did not affect the yield per plant or spike. Under this treatment the yield and quality of the grain surpassed that under either nitrogen or potash.

## CORRELATIONS

In order to determine what relation might exist between the total number of spikelets per spike and the number of sterile spikelets, the readings constituting the averages for the 25 spikes of each variety were arranged in correlation tables. The beardless varieties form one table and the bearded the other. Thus, the readings were the average of each variety and the array or distribution in the table was made up of varieties. The data were secured from the plants in hills. Since the number of spikelets per spike in a large measure determines the length of spike, the relation found will be closely associated with the length of the spike. In Table V, which includes the beardless varieties, the coefficient of correlation between the number of sterile spikelets and the total number of spikelets is $0.543 \pm 0.054$. The bearded varieties show a correlation which is expressed as $r = 0.598 \pm 0.041$. It appears that the number of sterile spikelets per variety bears a direct positive correlation to the total number of spikelets or the length of head. The varieties with the shorter spikes have decidedly fewer sterile spikelets. The relation between the number of spikelets and the length of spike may not be close, inasmuch as there may be more or less range among varieties as to the condensation or closeness of the spikelets on the spike. However, the long spikes are made up of a relatively larger number of spikelets than the short ones, and the actual percentage of sterile spikelets may be smaller in the long spikes, as will be pointed out later.

TABLE V.—*Correlation between the number of sterile spikelets and the total number of spikelets in beardless and bearded varieties of wheat*

BEARDLESS VARIETIES[1]

| Number of sterile spikelets. | 12 to 13. | 13 to 14. | 14 to 15. | 15 to 16. | 16 to 17. | 17 to 18. | 18 to 19. | 19 to 20. | Total. |
|---|---|---|---|---|---|---|---|---|---|
| 1 to 2 | ..... | ..... | ..... | 1 | 1 | 1 | ..... | ..... | 3 |
| 2 to 3 | ..... | 2 | 8 | 14 | 7 | 1 | ..... | ..... | 32 |
| 3 to 4 | ..... | ..... | 2 | 10 | 12 | 7 | 3 | 1 | 35 |
| 4 to 5 | ..... | ..... | ..... | ..... | 1 | 4 | 3 | 1 | 9 |
| Total | ..... | 2 | 10 | 25 | 21 | 13 | 6 | 2 | 79 |

BEARDED VARIETIES[2]

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 to 2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0 |
| 2 to 3 | 4 | 2 | 6 | 6 | ..... | ..... | ..... | ..... | 18 |
| 3 to 4 | ..... | 16 | 15 | 18 | 6 | ..... | 3 | ..... | 58 |
| 4 to 5 | ..... | ..... | 1 | 7 | 4 | 11 | 4 | ..... | 27 |
| 5 to 6 | ..... | ..... | 1 | ..... | ..... | ..... | 5 | ..... | 6 |
| Total | 4 | 18 | 23 | 31 | 10 | 11 | 12 | ..... | 109 |

[1] $r = 0.543 \pm 0.054$. [2] $r = 0.598 \pm 0.041$.

## CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND OTHER CHARACTERS OF THE WHEAT PLANT

For the purpose of studying the relationship between the percentage of sterile spikelets per plant and other characters, 300 plants of the variety Velvet Chaff were pulled, dried, and later carefully measured. The plants had been grown by the centgener method, 6 inches apart each way. The percentage of sterile spikelets was used rather than the actual number, for the reason that the length of spikes, which determines the number of spikelets, varies so greatly. The measurements of length were taken in centimeters and those of weight in milligrams. Biometrical data were secured for the statistical relationship between the percentage of sterile spikelets per plant and (1) the number of culms per plant; (2) the yield of grain per plant; (3) the yield of grain per spike; (4) the length of the culm; (5) the length of the spike; (6) the average weight of the kernel; and (7) the number of spikelets. In the above determinations the plant was used as a unit, the value for each character being determined by taking the average of the respective readings.

### CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS PER PLANT AND THE NUMBER OF CULMS PER PLANT

An inspection of Table VI shows only a slight degree of correlation between the percentage of sterile spikelets and the number of culms, which is negative. The coefficient of correlation is $-0.076 \pm 0.039$. Evidently there exists no appreciable relationship between the percentage of

sterile spikelets and the number of tillers per plant. The less vigorous plants, indicated by the smaller number of tillers per plant, do not show a higher percentage of sterile spikelets than the more thrifty plants.

TABLE VI.—*Correlation between the percentage of sterile spikelets per plant and the number of tillers per plant in wheat*[1]

| Percentage of sterile spikelets per plant. | Number of tillers per plant. | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | |
| 0 to 3 | 1 | 2 | .... | 1 | .... | 1 | .... | 1 | .... | .... | .... | .... | .... | 6 |
| 3 to 7 | 2 | 2 | 13 | 4 | 7 | 5 | 8 | 2 | 1 | 1 | 1 | .... | .... | 46 |
| 7 to 11 | 2 | 3 | 4 | 15 | 19 | 16 | 14 | 7 | 2 | .... | 1 | 1 | 1 | 85 |
| 11 to 15 | .... | 10 | 14 | 20 | 11 | 11 | 14 | 6 | 3 | 4 | 1 | 1 | .... | 95 |
| 15 to 19 | 1 | 2 | 7 | 10 | 11 | 4 | 5 | 2 | 1 | 1 | 2 | 1 | 1 | 48 |
| 19 to 23 | 2 | 1 | 4 | 1 | 2 | 1 | .... | .... | .... | .... | .... | .... | .... | 11 |
| 23 to 27 | .... | 1 | 4 | 2 | .... | .... | .... | .... | .... | .... | .... | .... | .... | 7 |
| 27 to 31 | .... | 1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 1 |
| 31 to 35 | .... | .... | .... | .... | .... | .... | 1 | .... | .... | .... | .... | .... | .... | 1 |
| Total | 8 | 22 | 46 | 53 | 50 | 38 | 42 | 18 | 7 | 6 | 5 | 3 | 2 | 300 |

[1] $r = -0.0756 \pm 0.0387$.

## CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND THE YIELD OF GRAIN PER PLANT

Between the percentage of sterile spikelets and the yield of grain per plant (Table VII) the coefficient of correlation is negative, $-0.306 \pm 0.035$. This correlation is fairly high and though expressed negatively indicates that the higher yielding plants have a smaller percentage of sterile spikelets than those of low yield.

TABLE VII.—*Correlation between the percentage of sterile spikelets per plant and the yield of grain per plant in wheat*[1]

| Percentage of sterile spikelets per plant. | Yield of grain per plant (in milligrams). | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 to 500 | 500 to 1,000 | 1,000 to 1,500 | 1,500 to 2,000 | 2,000 to 2,500 | 2,500 to 3,000 | 3,000 to 3,500 | 3,500 to 4,000 | 4,000 to 4,500 | 4,500 to 5,000 | 5,000 to 5,500 | 5,500 to 6,000 | 6,000 to 6,500 | 6,500 to 7,000 | |
| 0 to 3 | .... | 1 | 2 | 2 | .... | .... | .... | .... | .... | .... | 1 | .... | .... | .... | 6 |
| 3 to 7 | 1 | 5 | 7 | 6 | 6 | 7 | 2 | 5 | 2 | 3 | 1 | 1 | .... | .... | 46 |
| 7 to 11 | 2 | 3 | 12 | 15 | 23 | 10 | 10 | 4 | .... | 2 | 1 | 1 | 1 | 1 | 85 |
| 11 to 15 | 6 | 16 | 20 | 16 | 9 | 13 | 5 | 3 | 4 | 1 | .... | 1 | .... | 1 | 95 |
| 15 to 19 | 7 | 8 | 12 | 4 | 4 | 7 | 2 | .... | 1 | 2 | .... | .... | .... | 1 | 48 |
| 19 to 23 | 5 | 3 | 1 | 1 | 1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | 11 |
| 23 to 27 | 2 | 5 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 7 |
| 27 to 31 | 1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 1 |
| 31 to 35 | .... | 1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 1 |
| Total | 24 | 42 | 54 | 44 | 43 | 37 | 19 | 12 | 7 | 8 | 3 | 3 | 1 | 3 | 300 |

[1] $r = -0.3057 \pm 0.0353$.

## CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND THE AVERAGE YIELD OF GRAIN PER SPIKE

The average yield of grain per spike (Table VIII) was determined by dividing the total weight of grain per plant by the number of spikes per plant. The coefficient of correlation between this yield and the percentage of sterile spikelets is again negative, $-0.589 \pm 0.025$, which indicates a much closer relationship between the low percentage of sterile spikelets and yield of grain per spike than is shown between the same character and the yield per plant. There is a rather high correlation existing between the percentage of sterile spikelets and the yield of grain per spike.

TABLE VIII.—*Correlation between the percentage of sterile spikelets per plant and the average yield of grain per spike in wheat* [1]

| Percentage of sterile spikelets per plant. | Yield of grain per spike (in milligrams). | | | | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 to 100 | 100 to 150 | 150 to 200 | 200 to 250 | 250 to 300 | 300 to 350 | 350 to 400 | 400 to 450 | 450 to 500 | 500 to 550 | 550 to 600 | 600 to 650 | 650 to 700 | 700 to 750 | 750 to 800 | 800 to 850 | |
| 0 to 3 | ... | ... | .... | .... | .... | 1 | .... | .... | 1 | 2 | .... | ... | 1 | ... | 1 | ... | 6 |
| 3 to 7 | ... | ... | 1 | 1 | 2 | 2 | 3 | 7 | 8 | 7 | 6 | 1 | 1 | 6 | ... | 1 | 46 |
| 7 to 11 | ... | ... | .... | 4 | 9 | 7 | 17 | 12 | 14 | 13 | 6 | 2 | ... | 1 | ... | ... | 85 |
| 11 to 15 | ... | 2 | 6 | 11 | 13 | 17 | 16 | 13 | 8 | 3 | 4 | 2 | ... | ... | ... | ... | 95 |
| 15 to 19 | 2 | 3 | 2 | 11 | 8 | 8 | 9 | 2 | 1 | 1 | .... | 1 | ... | ... | ... | ... | 48 |
| 19 to 23 | 2 | 1 | 1 | 2 | 3 | 1 | 1 | .... | .... | .... | .... | ... | ... | ... | ... | ... | 11 |
| 23 to 27 | ... | ... | 2 | 4 | 1 | .... | .... | .... | .... | .... | .... | ... | ... | ... | ... | ... | 7 |
| 27 to 31 | ... | 1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | ... | ... | ... | ... | ... | 1 |
| 31 to 35 | ... | 1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | ... | ... | ... | ... | ... | 1 |
| Total | 4 | 8 | 12 | 33 | 36 | 36 | 46 | 34 | 32 | 26 | 16 | 6 | 2 | 7 | 1 | 1 | 300 |

[1] $r = -0.5888 \pm 0.0254$.

## CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS PER PLANT AND THE AVERAGE LENGTH OF CULM PER PLANT

In this case the average length of culm per plant (see Table IX) was found by taking the sum of the lengths of the culms of a plant in centimeters and dividing it by the number of culms. The correlation coefficient is $-0.448 \pm 0.031$. This is a rather high degree of correlation and is expressed as negative, although with reference to the relation of the two characters compared it means that the longer culms tend to form a lower percentage of sterile spikelets. This is what might be expected, since the yield of grain per spike is generally closely associated with the length of spike, and that in turn with the length of culm.

TABLE IX.—*Correlation between the percentage of sterile spiklets per plant and the average length of culm in wheat* [1]

| Percentage of sterile spikelets per plant. | Length of culm (in centimeters). | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 60 to 65 | 65 to 70 | 70 to 75 | 75 to 80 | 80 to 85 | 85 to 90 | 90 to 95 | 95 to 100 | 100 to 105 | 105 to 110 | 110 to 115 | 115 to 120 | |
| 0 to 3 | .... | .... | .... | .... | .... | 1 | 2 | 3 | .... | .... | .... | .... | 6 |
| 3 to 7 | .... | .... | .... | .... | 5 | 6 | 7 | 9 | 12 | 5 | 1 | 1 | 46 |
| 7 to 11 | .... | .... | .... | 3 | 11 | 13 | 17 | 15 | 19 | 6 | 1 | .... | 85 |
| 11 to 15 | .... | 1 | 2 | 6 | 16 | 21 | 21 | 15 | 9 | 3 | 1 | .... | 95 |
| 15 to 19 | .... | 2 | .... | 7 | 13 | 9 | 8 | 7 | .... | 2 | .... | .... | 48 |
| 19 to 23 | 1 | .... | 1 | 1 | 4 | 2 | 2 | .... | .... | .... | .... | .... | 11 |
| 23 to 27 | .... | 1 | 1 | 1 | 1 | 3 | .... | .... | .... | .... | .... | .... | 7 |
| 27 to 31 | .... | 1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 1 |
| 31 to 35 | .... | .... | .... | 1 | .... | .... | .... | .... | .... | .... | .... | .... | 1 |
| Total | 1 | 5 | 4 | 19 | 50 | 55 | 57 | 49 | 40 | 16 | 3 | 1 | 300 |

[1] $r = -0.4482 \pm 0.0316$.

## CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND THE LENGTH OF SPIKE PER PLANT

The average length of spike per plant was determined by dividing the sum of the lengths of the spikes per plant by the number of spikes. The calculations were expressed in centimeters. The coefficient of correlation between these two characters is $-0.451 \pm 0.031$ (see Table X). Since the longest spikes usually occupy the longest culms, we should expect the same relationship between the length of spike and percentage of sterile spikelets as was found between the latter character and the length of culm (see Table IX). There is a very close relation, the coefficient of correlation with the culm being $0.448 \pm 0.031$, a difference of 0.003 between the two coefficients.

TABLE X.—*Correlation between the percentage of sterile spikelets per plant and the average length of spike in wheat* [1]

| Percentage of sterile spikelets per plant. | Length of spike (in centimeters). | | | | | | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5.4 to 5.8 | 5.8 to 6.2 | 6.2 to 6.6 | 6.6 to 7 | 7 to 7.4 | 7.4 to 7.8 | 7.8 to 8.2 | 8.2 to 8.6 | 8.6 to 9 | 9 to 9.4 | 9.4 to 9.8 | 9.8 to 10.2 | 10.2 to 10.6 | 10.6 to 11 | 11 to 11.4 | 11.4 to 11.8 | 11.8 to 12.2 | 12.2 to 12.6 | 12.6 to 13 | |
| 0 to 3 | ... | ... | ... | ... | ... | ... | ... | 1 | 1 | ... | ... | ... | 2 | 2 | ... | ... | ... | ... | ... | 6 |
| 3 to 7 | ... | ... | ... | ... | ... | ... | 1 | 3 | 5 | 5 | 12 | 8 | 6 | 4 | ... | 2 | ... | ... | ... | 46 |
| 7 to 11 | ... | ... | ... | ... | ... | 3 | 4 | 6 | 19 | 14 | 17 | 14 | 5 | 2 | ... | ... | ... | 1 | ... | 85 |
| 11 to 15 | 1 | ... | 1 | 2 | 3 | 11 | 11 | 12 | 17 | 15 | 10 | 4 | 3 | 1 | 2 | 1 | ... | ... | 1 | 95 |
| 15 to 19 | ... | 1 | ... | ... | 2 | 3 | 12 | 8 | 4 | 8 | 5 | 2 | 1 | ... | 1 | 1 | ... | ... | ... | 48 |
| 19 to 23 | ... | ... | 1 | 1 | ... | 2 | 4 | 1 | ... | 1 | ... | ... | ... | 1 | ... | ... | ... | ... | ... | 11 |
| 23 to 27 | ... | ... | 1 | 1 | 3 | 1 | ... | ... | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 7 |
| 27 to 31 | ... | ... | ... | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 31 to 35 | ... | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| Total | 1 | 1 | 3 | 5 | 8 | 21 | 32 | 31 | 46 | 43 | 45 | 28 | 17 | 10 | 3 | 4 | 0 | 1 | 1 | 300 |

[1] $r = 0.4515 \pm 0.0310$.

## CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND THE AVERAGE WEIGHT OF KERNEL

To get the average weight of kernel per plant the total weight of kernels per plant was divided by the number of kernels and the result expressed in milligrams. The coefficient of correlation is $-0.421 \pm 0.032$ (see Table XI). This indicates a decided tendency for the heavier kernels to be associated with a low percentage of sterile spikelets. This is in accord with the relations found to exist between the length of culm and spike and the percentage of sterile spikelets. The more vigorous plants, as indicated by an increased length of culm and spike, generally bear kernels of a larger size. Hence, the correlation between the percentage of sterile spikelets and the weight of kernel—in other words, the quality of the grain—is in the same direction and approximates the other coefficients very closely.

TABLE XI.—*Correlation between the percentage of sterile spikelets per plant and the average weight of the kernel in wheat* [1]

| Percentage of sterile spikelets per plant. | Weight of kernel (in milligrams). | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 to 4 | 4 to 6 | 6 to 8 | 8 to 10 | 10 to 12 | 12 to 14 | 14 to 16 | 16 to 18 | 18 to 20 | 20 to 22 | 22 to 24 | 24 to 26 | |
| 0 to 3 | .... | .... | .... | .... | .... | .... | 1 | 3 | 1 | .... | 1 | .... | 6 |
| 3 to 7 | .... | .... | .... | 2 | 2 | 8 | 4 | 11 | 7 | 8 | 2 | 2 | 46 |
| 7 to 11 | .... | .... | .... | 1 | 6 | 17 | 15 | 26 | 13 | 4 | 1 | 2 | 85 |
| 11 to 15 | .... | 1 | 3 | 6 | 13 | 11 | 29 | 13 | 13 | 5 | .... | 1 | 95 |
| 15 to 19 | 1 | 1 | 1 | 6 | 7 | 15 | 10 | 5 | 1 | 1 | .... | .... | 48 |
| 19 to 23 | .... | .... | 3 | 1 | 3 | 1 | 1 | 2 | .... | .... | .... | .... | 11 |
| 23 to 27 | .... | .... | .... | .... | 2 | 3 | 2 | .... | .... | .... | .... | .... | 7 |
| 27 to 31 | .... | .... | 1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | 1 |
| 31 to 35 | .... | .... | .... | 1 | .... | .... | .... | .... | .... | .... | .... | .... | 1 |
| Total | 1 | 2 | 8 | 17 | 33 | 55 | 62 | 60 | 35 | 18 | 4 | 5 | 300 |

[1] $r = -0.4209 \pm 0.0320$.

## CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS PER PLANT AND THE AVERAGE NUMBER OF SPIKELETS PER SPIKE PER PLANT

A relationship is shown below between the percentage of sterile spikelets per plant and the total number of spikelets per plant. The coefficient of correlation is low, $-0.152 \pm 0.037$ (see Table XII). There is only a slight tendency for plants with a low percentage of sterile spikelets to be associated with a large number of spikelets per plant. As the number of spikelets determines to a large extent the length of the spike, it would be supposed that a greater correlation would exist between the number of spikelets and the percentage of sterile spikelets. This may be explained by the fact that the total number of spikelets includes both fertile and sterile spikelets. Also, there may be more or less variation in the condensation of the spikelets which go to make up the spike.

TABLE XII.—*Correlation between the percentage of sterile spikelets per plant and the average number of spikelets per spike per plant in wheat* [1]

| Percentage of sterile spikelets per plant. | Number of spikelets per spike. | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| 0 to 3 | .... | .... | .... | 1 | .... | 1 | .... | 3 | .... | 1 | .... | .... | .... | 6 |
| 3 to 7 | .... | .... | .... | 1 | 7 | 5 | 14 | 10 | 9 | .... | .... | .... | .... | 46 |
| 7 to 11 | .... | .... | 2 | 4 | 7 | 4 | 26 | 27 | 15 | .... | .... | .... | .... | 85 |
| 11 to 15 | .... | .... | 1 | 5 | 20 | 20 | 20 | 18 | 7 | 1 | 3 | .... | .... | 95 |
| 15 to 19 | 1 | .... | 1 | 1 | 4 | 12 | 10 | 12 | 6 | .... | .... | .... | 1 | 48 |
| 19 to 23 | .... | 1 | .... | 3 | .... | 3 | 1 | 1 | 1 | 1 | .... | .... | .... | 11 |
| 23 to 27 | .... | 1 | 1 | .... | 1 | 2 | 2 | .... | .... | .... | .... | .... | .... | 7 |
| 27 to 31 | .... | .... | .... | .... | .... | 1 | .... | .... | .... | .... | .... | .... | .... | 1 |
| 31 to 35 | .... | .... | .... | .... | .... | 1 | .... | .... | .... | .... | .... | .... | .... | 1 |
| Total | 1 | 2 | 5 | 15 | 39 | 49 | 73 | 71 | 38 | 3 | 3 | 0 | 1 | 300 |

[1] $r = -0.1524 \pm 0.0375$.

TABLE XIII.—*Variation constants in wheat*

| Plant as the unit. | Mean. | Standard deviation. | Coefficient of variation. |
|---|---|---|---|
| Sterile spikelets....per cent.. | 11.73 ± 0.198 | 5.105± 0.141 | 43.51 ±1.406 |
| Number of tillers per plant.... | 5.193± .091 | 2.336± .064 | 44.98 ±1.468 |
| Yield of grain per plant.mgm.. | 2,048.333±51.125 | 1,312.821±36.157 | 64.09 ±2.381 |
| Yield of grain per spike.mgm.. | 379.833± 5.475 | 140.599± 3.872 | 37.02 ±1.151 |
| Length of culm.....cm.. | 91.417± .366 | 9.411± .259 | 10.29 ± .286 |
| Length of spike.....cm.. | 9.019± .043 | 1.113± .031 | 12.34 ± .344 |
| Weight of kernel.....mgm.. | 15.033± .151 | 3.881± .107 | 25.82 ± .756 |
| Number of spikelets per spike. | 17.857± .065 | 1.688± .046 | 9.335± .257 |

| Characters. | Coefficient of correlation. |
|---|---|
| Sterile spikelets and number of tillers per plant..... | −0.075±0.038 |
| Sterile spikelets and yield of grain per plant.....mgm.. | − .306± .035 |
| Sterile spikelets and average yield of grain per spike.....mgm.. | − .589± .024 |
| Sterile spikelets and average length of culm per plant.....cm.. | − .448± .031 |
| Sterile spikelets and average length of spike.....cm.. | − .451± .031 |
| Sterile spikelets and weight of kernel per plant.....mgm.. | − .421± .032 |
| Sterile spikelets and the average number of spikelets per spike, per plant..... | − .152± .037 |

## SUMMARY

(1) The number of sterile spikelets per spike in wheat is directly affected by the rate of seeding or the spacing of the plants. The more space allowed each plant the smaller the number of sterile spikelets on each spike.

(2) The bearded varieties of wheat as a class have a higher percentage of sterile spikelets than the beardless varieties. Of the 188 varieties

examined the smallest number of sterile spikelets was found on a beardless variety and the largest number on a bearded variety.

(3) Early seeding seems to increase the percentage of sterile spikelets on each spike. Wheat seeded very late had the smallest percentage of sterile spikelets.

(4) The application of nitrogen alone as a fertilizer produced the lowest percentage of sterile spikelets. Phosphoric acid singly gave the highest percentage of sterile spikelets, while potash was intermediate as to the percentage of sterile spikelets. Where two elements of fertilizers were combined, phosphoric acid and potash gave the highest percentage of sterile spikelets, with nitrogen and phosphoric acid next and nitrogen and potash last. In every instance the check or untreated plots gave a lower percentage of sterile spikelets than those treated with a complete fertilizer.

(5) There is a distinct correlation between the length of spike as expressed by the number of spikelets and the number of sterile spikelets. As the number of spikelets per spike increases (in other words, the length of spike), the number of sterile spikelets becomes greater. That is, varieties with the shorter spikes tend toward a smaller number of sterile spikelets than the varieties with the longer spikes. However, the percentage of sterile spikelets per spike may be greater among the varieties with the shorter spikes, as was shown to be the case where spikes of varying lengths within a single variety were examined.

(6) There is only a very slight correlation between the percentage of sterile spikelets and the number of tillers to each plant.

(7) The yield of grain per plant is correlated to a fair degree with a low percentage of sterile spikelets.

(8) The weight of the kernel or quality of grain is correlated to a considerable degree with a low percentage of sterile spikelets.

(9) The yield of grain per spike, the length of spike, and the length of culm are strongly correlated with a low percentage of sterile spikelets.

(10) There is a slight correlation between the average number of spikelets per spike and a low percentage of sterile spikelets.

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## PLATE XXVII

Comparison of the number of sterile spikelets on bearded and beardless varieties of wheat:

On the left two heads of a bearded variety of wheat showing a large number of sterile spikelets. On the right two heads of a beardless variety showing comparatively few sterile spikelets. Both varieties were grown the same year under like conditions of soil and treatment.

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# EFFECT OF COLD-STORAGE TEMPERATURES UPON THE PUPÆ OF THE MEDITERRANEAN FRUIT FLY[1]

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## INTRODUCTION

The use to which cold-storage temperatures may be put as an aid in offsetting the disastrous results of attack by the Mediterranean fruit fly, *Ceratitis capitata* Wied., has already been made the subject of discussion by the writers.[2] In their paper, however, data on the effect of various ranges of temperatures used in commercial cold-storage plants upon the eggs and larval instars only are given. So far as the writers have been able to determine, fruits of almost any variety commonly held in storage are held at temperatures varying from 32° to 45° F., with preference shown to a range of 32° to 36°. The effect upon over 26,000 eggs and 60,000 larvæ of different temperatures, including 32°, 32° to 33°, 33° to 34°, 34° to 36°, 36°, 36° to 40°, 38° to 40°, and 40° to 45°, indicate that no eggs or larvæ survive refrigeration for seven weeks at 40° to 45°, for three weeks at 33° to 40°, or for two weeks at 32° to 33°.

While the greatest danger in the spread of this pest from one country to another lies in the transportation of the larvæ within fruits, there are certain data on record which prove that this pest may be carried long distances in the pupal stage and arrive at its destination in a condition to produce infestation. A fruit-fly pupa (species unknown) was found at Auckland, New Zealand, in soil about the roots of plants imported from Australia.[3] In 1914, Sasscer[4] records the discovery in Washington, D. C., of living pupæ of the papaya fruit fly (*Toxotrypana curvicauda* Gerst.) in a package containing an unknown vine from Mexico. In

[1] The writers wish to acknowledge the assistance given them by Mr. H. F. Willard in obtaining the data recorded in this and in their previous paper. To obtain these data has necessitated much prolonged tedious work extending over three years. In securing the data during 1915, Mr. Willard has not only greatly assisted, but on several occasions during the absence of the writers has been entirely responsible not only for the completion of experiments already started, but for the starting of others.

[2] Back, E. A., and Pemberton, C. E. Effect of cold-storage temperatures upon the Mediterranean fruit fly. *In* Jour. Agr. Research, v. 5, no. 15, p. 657–666. 1916.

[3] Kirk, T. W. Fruit flies. New Zeal. Dept. Agr. Div. Biol. Bul. 22, p. 9. 1909.

[4] Sasscer, E. R. Important insect pests collected on imported nursery stock in 1914. *In* Jour. Econ. Ent., v. 8, no. 2, p. 268–270. 1915.

another instance the same investigator records finding a living adult of the olive fruit fly (*Dacus oleae* Rossi) and a dead adult of another species of fruit fly, apparently *Dacus semispharens* Becker. Both of these species were in a small package containing olive seed from Cape Town, South Africa, after having been en route 28 days. Sasscer states that according to Silvestri it requires from 47 to 49 days in Italy for the pupæ of the olive fruit fly to yield adults; hence, it is possible for this ruinous pest to enter the United States through the eastern ports as pupæ and reach the olive-growing sections of California before adults have emerged.

Such facts as these indicate that the Mediterranean fruit fly may be similarly transported, and emphasize the desirability of recorded data on the effect of cold-storage temperatures upon the pupal stages. Aside from the practical application in the future to quarantines regulating the shipment of fruits, the results given below throw considerable light on conditions governing the distribution of the pest, and help explain the varying severity of its ravages in countries having both semitropical and temperate fruit-growing regions.

## HISTORICAL REVIEW

Practically nothing has been published on the effect of cold-storage temperatures upon the pupæ of *Ceratitis capitata*. In 1908 Lounsbury [1] in South Africa reports that in removing fruit infested with *C. capitata* from refrigeration at 38° to 40° F. at the end of 21 and 27 days he found in each instance a single pupa, but that both proved to be dead. The experiments of the writers have demonstrated that these two pupæ were produced by larvæ which formed their puparia before the fruit was placed in storage, as larvæ do not form puparia at temperatures lower than 45° to 48° F.

In 1914 Newman,[2] in Western Australia, placed one box containing 50 newly formed puparia in each of four rooms held, respectively, at 32°, 36°, 45°, and 55° F. At the end of 34 days of refrigeration 25 pupæ were taken from each box held at 32° and 36°, and at the end of 70 days of refrigeration the remaining pupæ held at 32° and 36° and all held at 45° and 55° were removed to the laboratory. None of the pupæ removed yielded adults.

## EXPERIMENTAL WORK

Nearly all the experimental work with temperatures lower than 45° F. was carried on in a thoroughly modern three-story cold-storage plant. The temperatures of the rooms in this plant were held quite definitely within certain fixed ranges by hourly inspections made by the storage employees. One experiment was carried on in a second plant where, as indicated in the text, the temperature was subject to considerable fluc-

[1] Lounsbury, C. P. Report of the Government Entomologist, Cape of Good Hope, 1907, p. 56. 1908.

[2] Newman, L. J. Annual report of the officer in charge of the insectary for the year ended June 30, 1914. *In* Ann. Rpt. Dept. Agr. West. Aust. 1914, p. 61. 1915.

tuation. The temperatures 49° to 51°, 52° to 56°, and 54° to 57° were not obtainable in the Honolulu cold-storage plants, hence in experiments at these temperatures ordinary refrigerators were used, as indicated. Usually pupæ of all ages from 1 to 9 or 10 days were obtained for each experiment, in order that varying effects upon pupæ in different stages of development might be noted. The pupæ were sifted from sand beneath host fruits and placed in storage either in bulk of several thousand in large jars or, as was more usual, in smaller lots of from one to several hundreds in vials about 1 inch in diameter and stoppered with cotton. Pupæ were not placed in or on damp sand or soil, as early experimental work indicated no advantage from this treatment when pupæ are subjected to cold-storage temperatures. The humidity of the storage rooms varied between 80° and 91°. After refrigeration the pupæ were removed to the laboratory, where they were daily observed for emergence records.

The term "pupa" is used to designate that period in the life history between the formation of the puparium by the larva and the emergence of the adult.

TEMPERATURE, 32° F.—Of the 13,900 pupæ of all ages subjected to refrigeration at a temperature varying less than half a degree either above or below 32° F. during the experiment, none survived more than 10 days. In Table I are recorded the results of observations on pupæ refrigerated from 2 to 10 days.

TABLE I.—*Effect upon Mediterranean fruit-fly pupæ of refrigeration at 32° F. for from 2 to 10 days*

| Age of pupæ on entering storage. | Number of pupæ yielding adults after removal to normal temperature after refrigeration for— | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 days. | 3 days. | 4 days. | 5 days. | 6 days. | 7 days. | 8 days. | 9 days. | 10 days. |
| 1 day | 15 | 6 | 3 | 0 | 1 | 0 | 0 | 0 | 0 |
| 2 days | 20 | 28 | 20 | 17 | 15 | 4 | 0 | 0 | 0 |
| 3 days | 32 | 21 | 22 | 19 | 4 | 4 | 3 | 1 | 0 |
| 4 days | 18 | 17 | 11 | 9 | 1 | 0 | 0 | 0 | 0 |
| 5 days | 28 | 18 | 3 | 1 | 1 | 1 | 0 | 0 | 0 |
| 6 days | 29 | 27 | 16 | 6 | 1 | 0 | 0 | 0 | 0 |
| 7 days | 48 | 33 | 13 | 5 | 2 | 4 | 0 | 0 | 0 |
| 8 days | 52 | 39 | 17 | 7 | 2 | 0 | 0 | 0 | 0 |
| 9 days | 51 | 33 | 21 | 12 | 4 | 0 | 0 | 0 | 0 |

Each lot removed after from 2 to 8 days of refrigeration contained 100 pupæ; hence, the number of pupæ yielding adults represents also the percentage of survival. Very few pupæ survived refrigeration at this temperature for longer than one week. Thus only 3 three-day-old pupæ out of 900 pupæ of all ages survived refrigeration for 8 days, and only 1 three-day-old pupa survived refrigeration for 9 days. While the data in Table I do not show it, the one surviving 9 days of refrigeration was one out of 300 of like age, and one out of 1,900 of all ages. Not one of 4,500 pupæ refrigerated for 10 days survived.

TEMPERATURES OF FROM 33° TO 34° F.—Only 3 out of 207 pupæ held at 33° to 34° F. for 4 days and at 43° to 45° F. for 8 additional days yielded adults.

TEMPERATURES OF FROM 33° to 36° F., AVERAGING 34°.—A total of over 27,097 pupæ were used in experiments to determine the effect of a temperature averaging about 34° F. but varying between 33° and 36° F. Only 1 seven-day-old pupa out of 228 of like age or 1,239 of all ages refrigerated for 16 days yielded an adult; 1,228, 1,164, 1,694, and 1,931 refrigerated for 18, 20, 22, and 25 days were dead on removal from storage. Only 3 out of 272 seven-day-old pupæ, or 1,472 pupæ of all ages, produced adults after refrigeration for 15 days, while only 8 out of 210 eight-day-old pupæ and 3 out of 220 seven-day-old pupæ, or but 11 out of 1,630 pupæ of all ages from one to eight days old when placed in storage, produced adults after refrigeration for 14 days. After refrigeration for 12 days, 12 eight-day-old pupæ, 11 seven-day-old pupæ, 2 six-day-old pupæ, and 8 one-day-old pupæ out of a total of 1,580 pupæ of all ages produced adults. From 1 to 30 adults emerged from lots of all ages of pupæ, totaling 1,519 forms, except from 126 five-day-old pupæ, after refrigeration for 11 days, but from 1 to 3 adults emerged from all lots yielding adults, except from the seven-day-old pupæ, which yielded 30 adults from a total of 265 pupæ.

Refrigeration of 1,685 pupæ of all ages for 9 days did not prove totally fatal to any age. Thus 85 out of 340 eight-day-old pupæ, and 88 out of 390 seven-day-old pupæ produced adults as compared with 3 four-day-old pupæ, 7 three-day-old pupæ, and 2 one-day-old pupæ out of a total of 475 pupæ.

Some adults emerged from lots of pupæ representing all ages on removal from storage after 2, 3, 4, 5, 6, 7, and 8 days of refrigeration. On these days an average of about 1,479 pupæ were removed from storage. The number of pupæ surviving is indicated by the data in Table II.

TABLE II.—*Effect upon pupæ of the Mediterranean fruit fly of refrigeration for from 1 to 8 days at 33° to 36° F.*

| Age of pupæ on entering storage. | Number of pupæ yielding adults after removal to normal temperature after refrigeration for— | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 days. | 3 days. | 4 days. | 5 days. | 6 days. | 7 days. | 8 days. |
| 1 day | 87 | 59 | 56 | 59 | 16 | 15 | 9 |
| 2 days | 41 | 12 | 25 | 6 | 8 | 2 | 6 |
| 3 days | 49 | 76 | 44 | 18 | 12 | 14 | 6 |
| 4 days | 27 | 47 | 26 | 11 | 4 | 5 | 3 |
| 5 days | 12 | 21 | 14 | 10 | 10 | 1 | 4 |
| 6 days | 10 | 15 | 17 | 10 | 5 | 8 | 2 |
| 7 days | 190 | 405 | 228 | 221 | 188 | 229 | 98 |
| 8 days | 129 | 153 | 434 | 146 | 216 | 150 | 91 |

The data in Table II are introduced to prove that refrigeration for from 1 to 8 days at this temperature is not fatal, and can not be depended

upon to kill all pupæ. While an average of about 1,479 pupæ were removed each day, the number of pupæ of each age is not known; hence no conclusion can be drawn regarding the relative effect of this refrigeration upon pupæ of different ages. The data given above for 9, 11, 12, 14, 15, and 16 days of refrigeration seem to indicate that the older pupæ withstand the effects of cold for a relatively longer period.

In a second experiment 50 out of 200 pupæ of all ages yielded adults after 4 days' refrigeration, and but 15 out of 207 pupæ held at 33° to 34° F. for 4 days and then at 43° to 45° F. for 3 additional days.

TEMPERATURES OF FROM 28° TO 40° F., AVERAGING 36°.—A total of 8,500 pupæ were placed in a cold-storage room the temperature of which was subject to far greater changes than are usual in commercial plants. While the temperature averaged about 36° F. a large portion of the time, for short periods during the night it dropped to freezing or even 28°, and during the heat of the day when supplies were being removed frequently rose to 38° to 40°. As each lot of the various ages from ½ to 9 days removed consisted of 100 pupæ, the numbers of pupæ yielding adults after the various numbers of days of refrigeration represent the percentages of survival. In Table III are recorded the effects of from 1 to 24 days of refrigeration on 6,800 pupæ.

TABLE III.—*Effect upon pupæ of refrigeration at temperatures varying between 28° and 40° F., but averaging about 36° F.*

| Age of pupæ on entering storage. | Number of pupæ yielding adults on removal to normal temperature after refrigeration for— | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 day. | 3 days. | 6 days. | 8 days. | 10 days. | 12 days. | 16 days. | 18 days. | 24 days. |
| ½ day | 81 | 2 | 0 | ..... | 0 | 0 | 0 | ..... | ..... |
| 1 day | 92 | 35 | 0 | ..... | 0 | ..... | ..... | 0 | ..... |
| 2 days | 62 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 days | 81 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 days | 55 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 days | 70 | 32 | 14 | 7 | 0 | 0 | 0 | 0 | 0 |
| 6 days | 94 | 21 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 days | 69 | 60 | 51 | 10 | 1 | 0 | 0 | 0 | 0 |
| 9 days | [a] 31 | [a] 27 | [a] 23 | 25 | 0 | 0 | ..... | ..... | ..... |

[a] Of these three lots of 100 pupæ each, 25, 17, and 16 pupæ, respectively, yielded adults July 8, or just before being placed in cold storage.

A total of 1,700 pupæ of various ages removed to normal temperature after refrigeration for 21, 27, 29, and 31 days were found to be dead.

TEMPERATURES OF FROM 38° TO 40° F.—A total of 52,604 pupæ were used in experiments to determine the effect upon pupæ of refrigeration at 38° to 40° F. An average of 1,860 pupæ of all ages were removed after refrigeration for 3, 4, 6, 7, 8, 10, 12, and 14 days. The number of pupæ for each age varied from 109 to 414 and averaged 234. The number of pupæ surviving refrigeration for from 3 to 14 days is recorded in Table IV.

TABLE IV.—*Effect upon Mediterranean fruit-fly pupæ of refrigeration for from 3 to 14 days at 38° to 40° F.*

| Age of pupæ on entering storage. | Number of pupæ yielding adults on removal to normal temperature after refrigeration for— | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 days. | 4 days. | 6 days. | 7 days. | 8 days. | 10 days. | 12 days. | 14 days. |
| 1 day | 110 | 13 | 5 | 1 | 5 | 1 | 0 | 1 |
| 2 days | 150 | 170 | 75 | 119 | 121 | 145 | 124 | 42 |
| 3 days | 62 | 58 | 81 | 23 | 4 | 6 | 4 | 4 |
| 4 days | 35 | 41 | 16 | 13 | 19 | 4 | 1 | 1 |
| 5 days | 91 | 86 | 52 | 63 | 24 | 9 | 12 | 6 |
| 6 days | 132 | 82 | 59 | 52 | 23 | 6 | 7 | 0 |
| 7 days | 235 | 117 | 114 | 121 | 96 | 60 | 32 | 22 |
| 8 days | 207 | 234 | 136 | 161 | 180 | 61 | 63 | 19 |

After refrigeration for 17 days only 3 out of 306 eight-day-old pupæ, 3 out of 384 seven-day-old pupæ, 3 out of 206 six-day-old pupæ, 1 out of 162 four-day-old pupæ, and 11 out of 374 two-day-old pupæ yielded adults, or only 21 out of 2,352 pupæ of all ages survived.

After refrigeration for 18 days only 9 out of 701 eight-day-old pupæ, 5 out of 250 seven-day-old pupæ, 1 out of 295 five-day-old pupæ, 1 out of 430 three-day-old pupæ, and 13 out of 400 two-day-old pupæ yielded adults; or only 29 out of 2,632 pupæ of all ages survived.

Nineteen days of refrigeration proved fatal to 1,911 pupæ of all ages except 2 out of 375 one-day-old pupæ. No living pupæ were found among 2,031 pupæ of all ages after refrigeration for 21 days, nor among 28,700 pupæ of all ages after refrigeration for 35 days.

TEMPERATURES OF FROM 40° TO 45° F.—In this experiment to determine the effect upon pupæ of temperatures ranging between 40° and 45° F., 8,800 pupæ from 1 to 10 days old were used. Each unit of pupæ contained 100 forms; hence, the numbers of pupæ yielding adults after refrigeration from 1 to 27 days as recorded in Table V represent the percentages of survival.

TABLE V.—*Effect upon Mediterranean fruit-fly pupæ of refrigeration at 40° to 45° F. for from 1 to 27 days*

| Age of pupæ on entering storage. | Number of pupæ yielding adults after removal to normal temperature after— | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 day. | 3 days. | 6 days. | 8 days. | 10 days. | 12 days. | 16 days. | 18 days. | 24 days. | 27 days. |
| 1 day | 22 | 78 | 15 | 3 | 1 | 13 | ..... | ..... | ..... | ..... |
| 2 days | 81 | 77 | 59 | ..... | 24 | ..... | ..... | 3 | ..... | ..... |
| 3 days | 69 | 91 | 1 | 1 | 2 | 0 | ..... | 0 | 0 | 0 |
| 5 days | 47 | 63 | 38 | 13 | 20 | 13 | 0 | 0 | 0 | 0 |
| 6 days | 66 | 82 | 72 | 58 | 49 | 31 | 5 | 0 | 0 | 0 |
| 7 days | 91 | 87 | 75 | ..... | 60 | 57 | ..... | ..... | ..... | ..... |
| 8 days | 55 | 60 | 44 | 54 | 27 | 16 | 4 | 1 | 1 | 1 |
| 9 days | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 10 days | [a] 17 | [a] 23 | [a] 21 | 8 | 1 | [a] 1 | ..... | 0 | ..... | ..... |

[a] Besides these, 18, 28, 30, and 10 pupæ, respectively, yielded adults just before pupæ were placed in cold storage.

It will be noted that only 9 out of 300 pupæ survived refrigeration for 16 days, while only 4 out of 500 and 1 out of 500 refrigerated for 18 and 24 days, respectively, survived. Three hundred pupæ refrigerated for 31 days and 200 refrigerated for 34 days were found dead on removal.

TEMPERATURES OF FROM 49° TO 51° F.—Temperatures ranging between 49° and 51° F. and averaging about 50° have proved most interesting of all, as these appear to be very close to the point below which the insect's activities cease. This temperature was secured by use of an ordinary refrigerator 42 by 34 by 18 inches. During the period from May to July, 1914, 31,700 pupæ were used in an experiment to determine the effect of this temperature upon pupal development. Pupæ in 15 lots, of ages ranging from 1 to 8 days, and averaging 3,523 pupæ for each of the 8 days represented, were held in storage for two months before removal. Frequent observations were made but no pupæ completed their development and yielded adults in storage. On removal to normal temperature all of the 31,700 pupæ were found dead.

The second lot of 7,800 pupæ placed in storage when 5 days old yielded a few adults. Thus, 9 out of 7,800 yielded 1, 2, 2, 3, and 1 adult in storage after refrigeration for 20, 23, 44, 46, and 47 days. In other words, it took these 9 pupæ from 20 to 47 days to accomplish the development in refrigeration which at an outdoor temperature at that season, July, 1914, would have taken only from 4 to 5 days.

TEMPERATURES OF FROM 52° TO 56° F.—Ten larvæ pupating in a refrigerator held at 52° to 56° F. yielded 2 and 1 adult in storage after refrigeration for 38 and 52 days, respectively. The remaining 7 pupæ died.

TEMPERATURES OF FROM 54° TO 57° F.—Temperatures of from 54° to 57° F. were obtained by using an ordinary refrigerator 46 by 27 by 18 inches. A total of 22,700 pupæ were used varying in age from ½ to 9 days. Not less than 1,400 pupæ, or more than 3,500 pupæ of any age, were used. In Table VI are recorded the reactions of 3,100 one-day-old pupæ to these temperatures.

From the data in Table VI it will be noted that 54° to 57° F. is not in all cases fatal to pupal development, although a high mortality occurs. Each outward date represents 100 pupæ. As the heavy line extending diagonally across the table indicates the dates on which pupæ were removed from refrigeration, and as the normal pupal development is completed at this season of the year at Honolulu in from 9 to 12 days, the data prove that development continues at this temperature as evidenced, first, by the rate of emergence of adults after the pupæ are removed from refrigeration up to the thirtieth day of refrigeration, and, secondly, by the emergence actually occurring within storage on the thirty-first day and up to the thirty-seventh day of refrigeration. Thus development was wholly completed and emergence had taken place at this temperature among pupæ removed from refrigeration after 37, 38, and 39 days.

TABLE VI.—*Effect upon 1-day-old Mediterranean fruit-fly pupæ of refrigeration at 54° to 57° F. Pupæ placed in refrigeration August 22, 1913*

| Outward date. | Date and emergence of adults. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | August. | | | | September. | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 28 | 29 | 30 | 31 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
| Aug. 24 | 1 | 0 | 0 | 10 | 32 | 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 26 | | 1 | 0 | 2 | 4 | 25 | 21 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 27 | | | | | | | 17 | 19 | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| 28 | | | | | | | | 29 | 36 | | | | | | | | | | | | | | | | | | | | | | | | |
| Sept. 3 | | | | | | | | | 1 | 0 | 0 | 15 | 14 | 1 | | | | | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | 1 | 24 | 17 | | | | | | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | 1 | 19 | 12 | | | | | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | | 14 | 15 | | | | | | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | | | | 6 | 16 | 4 | | | | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | | 1 | 0 | 0 | 23 | 22 | 1 | | | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | | | | | | 13 | 18 | | | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | | | | | | 2 | 22 | 18 | | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | | | | | | | 1 | 5 | 17 | 8 | | | | | | | | | | | |
| 13 | | | | | | | | | | | | | | | | | 1 | 0 | 0 | 11 | 23 | 4 | | | | | | | | | | |
| 14 | | | | | | | | | | | | | | | | | | | 1 | 13 | 28 | | | | | | | | | | | |
| 15 | | | | | | | | | | | | | | | | | | | 0 | 0 | 4 | 24 | 10 | | | | | | | | | |
| 16 | | | | | | | | | | | | | | | | | | | | | | 8 | 30 | 7 | | | | | | | | |
| 17 | | | | | | | | | | | | | | | | | | | | | | 1 | 3 | 11 | 13 | | | | | | | |
| 18 | | | | | | | | | | | | | | | | | | | | | | 1 | 2 | 21 | 19 | | | | | | | |
| 19 | | | | | | | | | | | | | | | | | | | | | | | 5 | 24 | 6 | | | | | | | |
| 20 | | | | | | | | | | | | | | | | | | | | | | | | 3 | 9 | 9 | | | | | | |
| 21 | | | | | | | | | | | | | | | | | | | | | | | | 4 | 10 | 7 | 6 | | | | | |
| 22 | | | | | | | | | | | | | | | | | | | | | | | | 1 | 6 | 15 | 9 | 5 | | | | |
| 23 | | | | | | | | | | | | | | | | | | | | | | | | 7 | 3 | 5 | 3 | 6 | | | | |
| 24 | | | | | | | | | | | | | | | | | | | | | | | | 1 | 7 | 3 | 5 | 6 | 3 | | | |
| 25 | | | | | | | | | | | | | | | | | | | | | | | 1 | 0 | 3 | 2 | 5 | 3 | 9 | 11 | | | |
| 26 | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | 9 | 8 | 7 | 4 | 2 | | |
| 27 | | | | | | | | | | | | | | | | | | | | | | | 1 | 0 | 0 | 1 | 6 | 2 | 2 | 1 | 3 | 3 | 2 |
| 28 | | | | | | | | | | | | | | | | | | 1 | 0 | 0 | 1 | 6 | 6 | 3 | 8 | 1 | 7 | 0 | 1 | 1 | | |
| 29 | | | | | | | | | | | | | | | | | | | | | | | | | 3 | 6 | 6 | 5 | 4 | 1 | | |
| 30 | | | | | | | | | | | | | | | | | | | | | | | | | 3 | 16 | 9 | 3 | 2 | 2 | 4 | 2 | |

Of 1,700 pupæ one-half day old when placed in refrigeration none emerged within storage until the twenty-fourth day of refrigeration, after which no data were secured. One hundred pupæ removed after 24 days of refrigeration produced 2, 27, and 5 adults 4, 5, and 6 days after removal, proving that even among these very young pupæ development was not completely arrested. Out of two lots of 100 pupæ each, 46 and 44 pupæ, respectively, refrigerated for 22 and 23 days, yielded adults 3, 4, and 5 days after removal from storage.

Data on file bring out an interesting fact that might be expected from the data in Tables VI and VII. The older the pupæ when placed in refrigeration, the quicker they develop and produce adults while in refrigeration. Thus in Table VII are recorded data on the development of six-day-old pupæ.

It will be noted that while a few one-day-old pupæ require a minimum of 31 days of refrigeration for development, 2 six-day-old pupæ com-

pleted their development and produced adults in storage on the fifth day of refrigeration, and that thereafter emergence of adults continued until all living pupæ yielded adults in storage by the end of the sixteenth day of refrigeration except 2, which yielded adults on the fourth day after removal after 16 days of storage. Data on 1,900 eight-day-old pupæ show that from 1 to 5 pupæ among each of 14 different lots of 100 completed their development and yielded adults on the second day of refrigeration, that an average of 43.5 per cent of 14 lots yielded adults on the third day, and that emergence of adults was completed by the seventh day except in one instance where 2 pupæ yielded adults in storage on the ninth and tenth days of refrigeration.

TABLE VII.—*The effect upon six-day-old Mediterranean fruit-fly pupæ of refrigeration at 54° to 57° F. Pupæ placed in refrigeration August 22, 1913*

| Outward date. | Date and emergence of adults. | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | August— | | | | | | | September— | | | | | | | | |
| | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Aug. 24 | .... | .... | 57 | 6 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| 26 | .... | .... | 1 | 45 | 29 | .... | .... | .... | 1 | .... | .... | .... | .... | .... | .... | .... | .... |
| 27 | .... | .... | .... | 2 | 37 | 0 | 22 | 0 | 1 | .... | .... | .... | .... | .... | .... | .... | .... |
| 28 | .... | .... | .... | .... | 4 | 0 | 67 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| Sept. 3 | .... | .... | .... | .... | 1 | 0 | 10 | 8 | 19 | 13 | 0 | 3 | .... | .... | .... | .... | .... |
| 4 | .... | .... | .... | .... | .... | .... | 13 | 13 | 13 | 7 | 11 | .... | .... | .... | .... | .... | .... |
| 5 | .... | .... | 0 | 0 | 0 | 0 | 13 | 13 | 12 | 12 | 12 | 2 | 3 | .... | .... | .... | .... |
| 6 | .... | .... | .... | 1 | 1 | 0 | 7 | 12 | 16 | 15 | 17 | 10 | 4 | .... | .... | .... | .... |
| 7 | .... | .... | 1 | 0 | 1 | 0 | 11 | 10 | 13 | 9 | 19 | 7 | 2 | 0 | 0 | 0 | 2 |
| 8 | .... | .... | 1 | 1 | 0 | 0 | 13 | 10 | 13 | 13 | 11 | .... | .... | .... | .... | .... | .... |
| 9 | .... | .... | 0 | 2 | 2 | 0 | 11 | 5 | 9 | 15 | 10 | 2 | .... | .... | .... | .... | .... |
| 10 | .... | .... | .... | 1 | 0 | 0 | 13 | 21 | 13 | 9 | 18 | 3 | 2 | .... | .... | .... | .... |
| 12 | .... | .... | 0 | 0 | 0 | 0 | 11 | 6 | 13 | 10 | 13 | 1 | 2 | 3 | .... | .... | .... |

Data on file covering observations on 1,600, 1,400, 1,700, 2,200, 3,300, 4,000, 3,100, 3,500, and 1,900 pupæ, 1, 2, 3, 4, 5, 6, 7, 8, and 9 days old, respectively, show a steady increase in the pupæ completing their development and yielding adults in refrigeration, and their tabulation shows a transition from the condition in Table VI through that of Table VII to the condition set forth for eight-day-old pupæ. One lot of two-day-old pupæ left in storage for 37 days yielded adults almost daily between the twenty-sixth and thirty-fifth days of refrigeration. Eight lots of three-day-old pupæ left in storage from 32 to 39 days yielded adults between the twenty-third and thirty-third days of refrigeration and none thereafter. Fourteen lots of four-day-old pupæ in storage from 25 to 39 days yielded adults between the sixteenth and twenty-seventh days of refrigeration and none thereafter. Eight lots of seven-day-old pupæ in storage from 13 to 21 days yielded adults between the second and eleventh days of refrigeration and none thereafter.

## CONCLUSION

From the data secured during experimental work reported on the foregoing pages, including observations on 173,318 pupæ of the Mediterranean fruit fly (*Ceratitis capitata* Wied.), it appears that no pupæ survive refrigeration for longer periods than is necessary to cause the death of eggs and larvæ in host fruits held at corresponding temperatures.

About 50° F. is the critical point below which development can not take place and below which death will follow if refrigeration is continued sufficiently long. At 49° to 51° only 9 out of 39,500 pupæ yielded adults in refrigeration 20 to 47 days after the inward date, while 3 out of 6 held at 52° to 56° yielded adults in refrigeration 38 to 52 days after the inward date. Many pupæ can complete their entire development in refrigeration at 54° to 57°, while higher temperatures, not considered here, merely retard development without causing noticeable mortality.

Pupæ can not withstand temperatures below 50° F. for prolonged periods of time. Only 3 and 1 pupa survived refrigeration for 8 and 9 days, respectively, at 32°, while none of 4,500 pupæ survived 10 days at this temperature. Refrigeration at a temperature averaging 34°, but ranging between 33° and 36°, proved fatal after the seventeenth day; 6,017 pupæ refrigerated at this temperature for 18 and 25 days yielded no adults, while the number to yield adults after refrigeration for 14 and 17 days was very small. No pupæ survived refrigeration at 28° to 40° but averaging 36°, for more than 10 days. A temperature of 38° to 40° proved fatal after the nineteenth day; 30,731 pupæ refrigerated for from 21 to 35 days failed to yield adults on removal to normal temperatures. After refrigeration at 40° to 45° pupæ from each of two lots removed after refrigeration for 24 and 27 days, respectively, yielded adults; 500 pupæ removed after refrigeration for from 31 to 34 days proved to be dead.

It does not seem safe to conclude that the age of the pupa has a direct bearing upon its ability to withstand the more ordinary ranges of cold-storage temperatures.

# EFFECT OF CLIMATIC FACTORS ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM

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## INTRODUCTION

The present experiments are a continuation of those carried out in 1914 (10)[1] on sorghum (*Sorghum vulgare*). In the latter a correlation was sought between the soil conditions, especially the supply of nitrogen, and the amount of the cyanogenetic glucosid (dhurrin) in the sorghum. It was found that on fertile soils nitrogenous fertilizer has no appreciable effect, but on poor soil added nitrogen may increase the amount of hydrocyanic acid, though only to a small extent. Since the evidence indicated that climate and variety may be more important factors than soil nitrogen in determining the amount of the glucosid in this plant, experiments were carried out during 1915 to study the effect of climatic conditions. It was thought that conditions of high or low temperature, much or little available water, slow or rapid growth, might affect the metabolism of sorghum sufficiently, not only to show the causes of the varying amount of dhurrin, but also to throw some light on the physiological function of this glucosid.

## EXPERIMENTAL WORK

Seeds of two varieties of sorghum were obtained. One was Early Amber, grown in Minnesota, and is designated in these experiments Variety N. The other was Southern Cane, a variety similar to the first, but grown in Missouri. It is designated Variety S. In order to secure as widely varying climatic conditions as possible, one-twentieth-acre plots of each variety were grown at four different State experiment stations. A brief description of each plot follows:

1. University Farm, St. Paul, Minn. Very fertile, black loam, fair drainage. Planted on June 3; sprouted on June 12; cultivated twice. Season very cold and wet; sorghum three or four weeks behind the normal in development; did not reach maturity, but was killed by frost in the soft dough stage.

2. Agricultural Experiment Station, Logan, Utah. Irrigation farming. Plots on McNiel farm, North Logan; the two varieties alternated with beans; soil a clay loam, rich in manure. Planted May 15; appeared aboveground on June 1; irrigated on July 9 and August 11; cultivated on June 10, June 17, July 1, July 13, and August 17. Rainfall up to June 10 was abnormally high, which kept the soil cold and retarded growth of crops. During the rest of the season optimum moisture content of soils

[1] Reference is made by number to "Literature cited," p. 272.

obtained. Sorghum made slow growth; leaves yellowish for first seven or eight weeks.

3. Agricultural Experiment Station, Manhattan, Kans. Plots grown on "creek bottom land," broken from native sod in 1913; drainage poor. Planted on June 15; appeared aboveground on June 22; cultivated twice. Sorghum 30 days slower in maturing than usual, owing to excessive rains.

4. Belle Fourche Experiment Farm, Newell, S. Dak. Dry farming. Planted on June 10; appeared aboveground on June 26; cultivated on July 22 and August 7; harvested on October 12. Season cold and wet; rainfall far above normal.

5. Belle Fourche Experiment Farm, Newell, S. Dak. Irrigation farming. Planted on May 31; appeared aboveground on June 26; cultivated on July 23 and August 10; irrigated on August 17; harvested on September 16, when plants were just headed out.

From the time when the plants were from 20 to 30 cm. in height, samples were taken every 10 days. They were usually cut between 9 and 12 a. m., although it has been found that the time of day makes no difference in the amount of hydrocyanic acid present. Plants were selected which represented the average of the plot on that date. For the first sample the whole plant was cut into 1-inch lengths and packed into a 600 c. c. friction-top tin can with 20 c. c. of 3 per cent alcoholic sodium hydrate and 2 c. c. chloroform for preservatives and sent to the Minnesota laboratory for analysis. For the other samples the leaves were cut off where they join the sheath, and the leaves and stalks were packed and analyzed separately. The weight of leaves and of stalks in the total sample cut was recorded. From the fourth sample on, cans of 1,600 c. c. capacity were used. An alkaline preservative was used so as to prevent any possible loss of hydrocyanic acid set free by enzymic activity. Alcohol instead of water was used as a solvent for the alkali, because it penetrates the plant tissues more readily. The chloroform prevented any fermentative changes. In the case of the South Dakota, Kansas, and Utah samples, from two to five days elapsed from the time the samples were cut till they were analyzed. In the case of the Minnesota samples, the fresh material was analyzed. In order to test the efficiency of the preservative, several samples from the Minnesota plots, representing the various stages of maturity of the samples outside of Minnesota, were analyzed for hydrocyanic acid before and after storage in cans, with the results given in Table I.

TABLE I.—*Efficiency of an alkaline preservative in preventing loss of hydrocyanic acid in sorghum*

| Preservative treatment. | Percentage of hydrocyanic acid in dry matter. | |
|---|---|---|
| | Fresh. | Preserved. |
| Preserved for four days with alcoholic sodium hydroxid and chloroform | 0.019 | 0.020 |
| Do | .026 | .029 |
| Preserved for eight days with alcoholic sodium hydroxid and chloroform | .009 | .009 |
| Do | .016 | .019 |

The differences noted are within the limits of accuracy of sampling and analyzing; hence, this method of preservation can safely be used on sorghum plants at least through the stages of maturity represented in these experiments.

About 50 gm. of the sample, after thorough mixing and fining with a knife, were used to determine the percentage of dry matter. For the determination of the hydrocyanic-acid content, from 50 to 70 gm. were ground in a food chopper, placed in an 800 c. c. Kjeldahl flask, together with 250 c. c. of 5 per cent tartaric acid, and distilled slowly into 10 c. c. of 2 per cent sodium hydroxid until the distillate was nearly 100 c. c. This completely hydrolizes the dhurrin and carries the hydrocyanic acid over into the alkaline distillate. The latter was made to 100 c. c. and aliquots used for the determination of hydrocyanic acid according to the method of Viehoefer and Johns (9). This method was found to be easier and more accurate than the thiocyanate method used in 1914.

The complete analytical results appear in Table II. The figures for the amount of hydrocyanic acid in the whole plant were computed from the relative proportion of leaves and stalks in each sample.

TABLE II.—*Hydrocyanic-acid content of sorghum from the various experimental plots*

[The percentage of hydrocyanic acid is reported on a dry-matter basis]

| Plot and sample No. | Date of sampling. | Age of plants since sprouting. | Plot N. | | | | Plot S. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Height of plants. | Percentage of hydrocyanic acid. | | | Height of plants. | Percentage of hydrocyanic acid. | | |
| | | | | Stalks. | Leaves. | Whole plant. | | Stalks. | Leaves. | Whole plant. |
| Minnesota: | | *Days.* | *Cm.* | | | | *Cm.* | | | |
| 1 | July 15 | 33 | 25 | ..... | ..... | 0.114 | 25 | ..... | ..... | 0.079 |
| 2 | July 24 | 42 | 44 | 0.026 | 0.031 | .028 | 48 | 0.028 | 0.035 | .032 |
| 3 | Aug. 3 | 52 | 68 | .018 | .021 | .019 | 65 | .015 | .031 | .026 |
| 4 | Aug. 13 | 62 | 90 | .012 | .008 | .009 | 88 | .023 | .011 | .016 |
| 5 | Aug. 24 | 73 | 135 | .000 | .004 | .002 | 133 | Trace. | .007 | .003 |
| 6 | Sept. 3 | 83 | 160 | Trace. | .004 | .001 | 160 | .000 | .004 | .001 |
| 7 | Sept. 13 | 93 | 188 | ..... | .001 | Trace. | 192 | ..... | .002 | Trace. |
| 8 | Sept. 23 | 103 | 205 | ..... | ..... | ..... | 206 | ..... | .003 | Do. |
| Utah: | | | | | | | | | | |
| 1 | July 19 | 49 | 36 | ..... | ..... | .034 | 36 | ..... | ..... | .040 |
| 2 | July 29 | 59 | 56 | .028 | .013 | .019 | 59 | .039 | .025 | .031 |
| 3 | Aug. 7 | 68 | 78 | .019 | .023 | .021 | 84 | .026 | .032 | .029 |
| 4 | Aug. 18 | 79 | 120 | Trace. | .022 | .009 | 116 | .007 | .034 | .020 |
| 5 | Aug. 28 | 89 | 161 | .000 | .026 | .008 | 160 | Trace. | .041 | .016 |
| 6 | Sept. 7 | 99 | 174 | ..... | ..... | ..... | 175 | ..... | ..... | ..... |
| 7 | Sept. 17 | 109 | 190 | ..... | ..... | ..... | 192 | ..... | ..... | ..... |
| Kansas: | | | | | | | | | | |
| 1 | July 16 | 24 | 18 | ..... | ..... | .016 | 26 | ..... | ..... | .014 |
| 2 | July 27 | 35 | 75 | .000 | .017 | .008 | 95 | .000 | .030 | .014 |
| 3 | Aug. 5 | 44 | 137 | .000 | .007 | .003 | 150 | .000 | .020 | .008 |
| 4 | Aug. 16 | 55 | 200 | ..... | ..... | ..... | 210 | ..... | ..... | ..... |
| 5 | Aug. 25 | 64 | 260 | ..... | ..... | ..... | 255 | ..... | ..... | ..... |
| Dakota (dry farming): | | | | | | | | | | |
| 1 | July 26 | 30 | 33 | ..... | ..... | .020 | 33 | ..... | ..... | .030 |
| 2 | Aug. 5 | 40 | 61 | .004 | .013 | .011 | 61 | .009 | .021 | .016 |
| 3 | Aug. 14 | 49 | 91 | .000 | .000 | .000 | 86 | .000 | .008 | .004 |
| 4 | Aug. 24 | 59 | 142 | .000 | .004 | .002 | 137 | .000 | .006 | .002 |
| 5 | Sept. 4 | 70 | 189 | ..... | ..... | ..... | 187 | ..... | ..... | ..... |
| 6 | Sept. 14 | 80 | 282 | ..... | ..... | ..... | 287 | ..... | ..... | ..... |
| Dakota (irrigation): | | | | | | | | | | |
| 1 | July 26 | 30 | 35 | ..... | ..... | .009 | 37 | ..... | ..... | .026 |
| 2 | Aug. 5 | 40 | 51 | .004 | .008 | .006 | 51 | Trace. | .009 | .005 |
| 3 | Aug. 14 | 49 | 96 | .000 | Trace. | Trace. | 91 | .000 | Trace. | Trace. |
| 4 | Aug. 24 | 59 | 134 | .000 | ...do.. | ...do... | 134 | .000 | .001 | Do. |
| 5 | Sept. 4 | 70 | 193 | ..... | ..... | ..... | 190 | ..... | ..... | ..... |

In figure 1 the percentages of hydrocyanic acid in the whole plant are plotted against the age in days. No noteworthy differences were noticed when the height of the plants was used instead of the age in days.

Figure 2 represents the growth curve of the various plots, where the height in centimeters is plotted against the age in days since sprouting.

In order to study the relation between climatological factors and the content of hydrocyanic acid, figures 3 and 4 were constructed. In

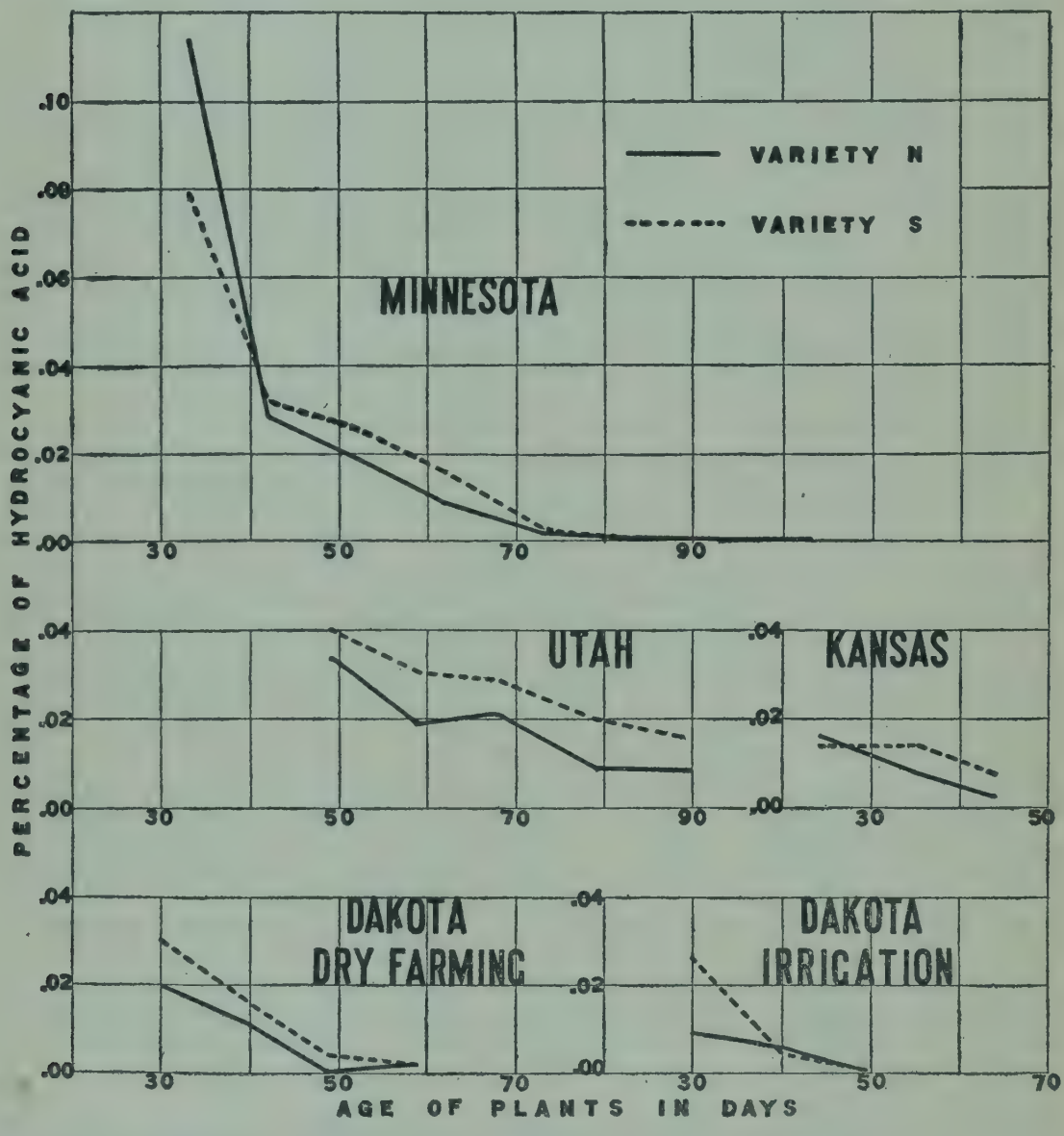


FIG. 1.—Curves showing the hydrocyanic-acid content of sorghum on the various plots. (Percentage of hydrocyanic-acid computed to dry-matter basis.)

figure 3 are plotted first the precipitation (in inches) during 15-day intervals; second, the temperature (degrees Fahrenheit), using averages for 10-day periods, and, third, the mean relative humidity (percentage) at 6 a. m., all for the five months May to September, inclusive. In figure 4 the history of each plot for the season is shown and includes the rainfall, temperature, and hydrocyanic-acid curves on the same graph. The dates for planting, sprouting, appearance of seed panicles, and irrigations are also shown.

## DISCUSSION OF RESULTS

The season of 1915 furnished some excellent extremes in weather conditions for this experiment. Figure 2 shows that, as regards temperature, the two more southern States, Kansas and Utah, form one pair, and South Dakota and Minnesota another, with approximately 10 degrees difference between them during the growing season. Of the two warmer stations, Utah had a low rainfall, and irrigation was resorted

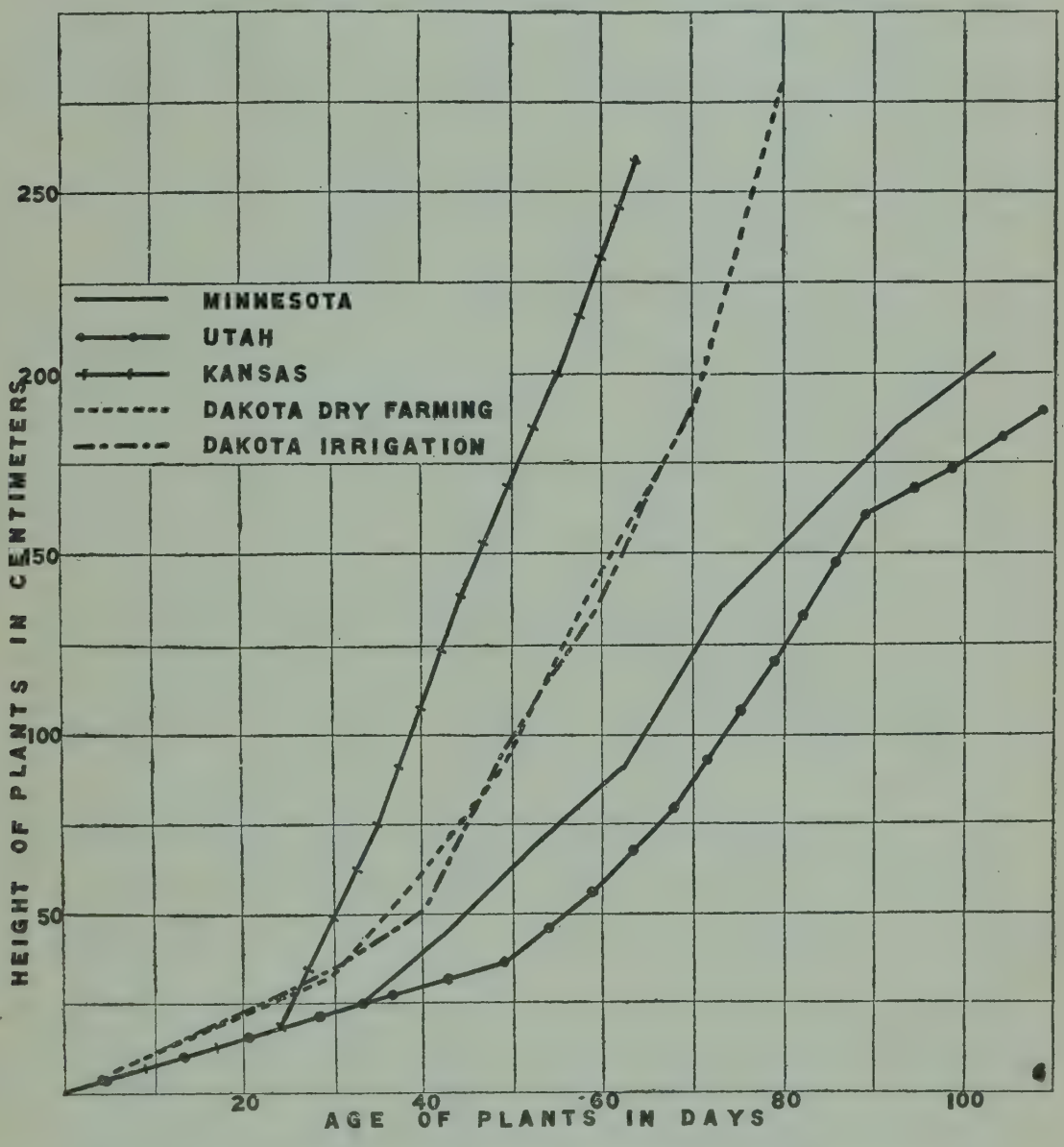


FIG. 2.—Curves showing the rate of growth of the sorghum on the various plots.

to; while Kansas had a very abundant rainfall, resulting even in flood conditions during May, June, and July. The two more northern stations had about the same rainfall for the first three months of the experiment, but during the period when the samples were taken the rainfall of South Dakota dropped below that of Minnesota. This was particularly the case during August. The 1915 rainfall of South Dakota was above normal, and as a result the plot on irrigation ground was irrigated only once.

The five plots differed rather widely in their rate of growth, as is shown in figure 4. The Utah plants were 48 days old before attaining the height required for first sample. During this time they looked yellow and unthrifty, owing to excess moisture and cool soil. Subsequently

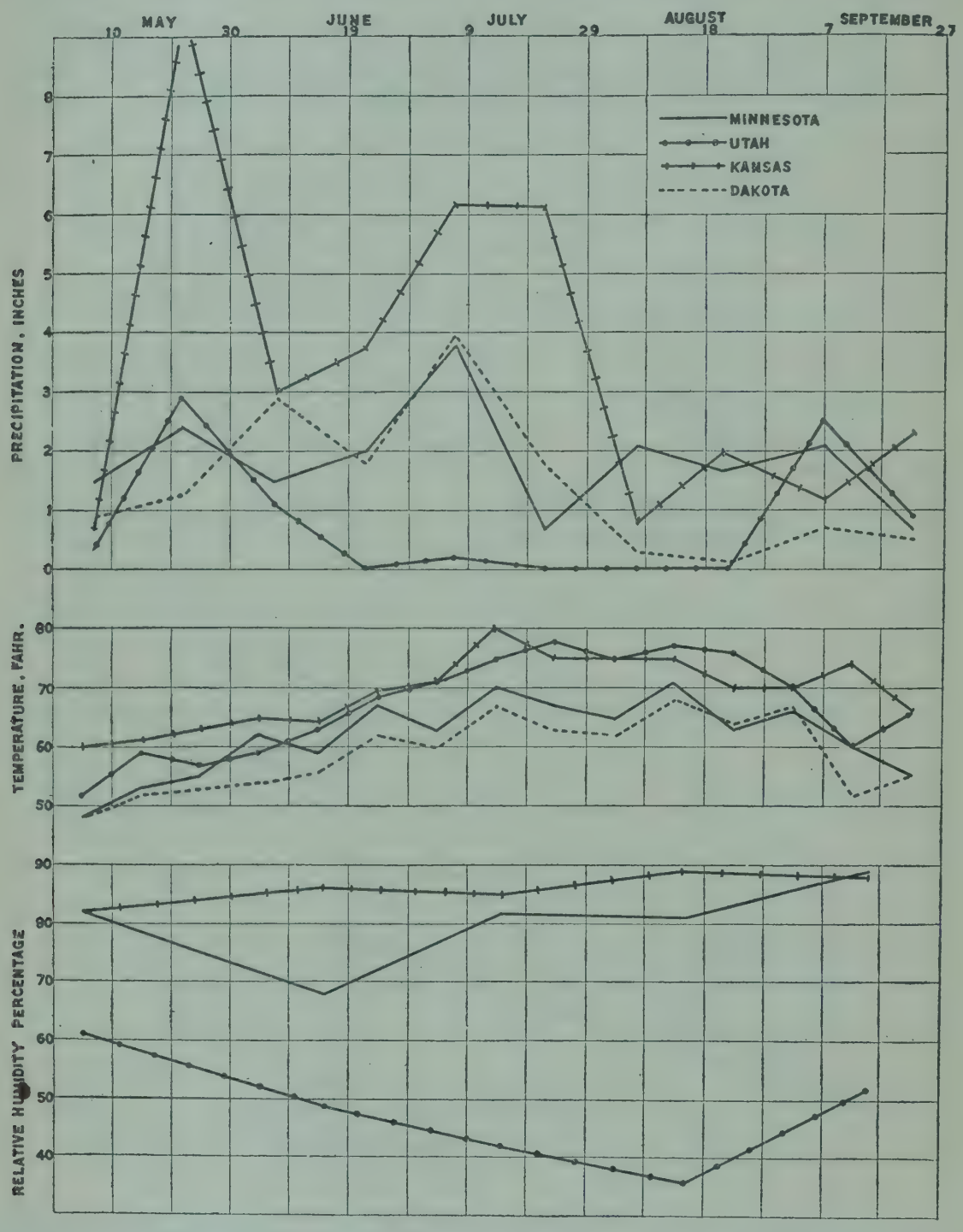


FIG. 3.—Curves showing the precipitation, temperature, and humidity relations at the various experiment stations during the growing season of 1915.

the plots grew nearly as fast as those at the other stations and gave a higher yield of dry cane at the end of the season than did the South Dakota plots, although the latter grew much taller. The Kansas plots grew the most rapidly.

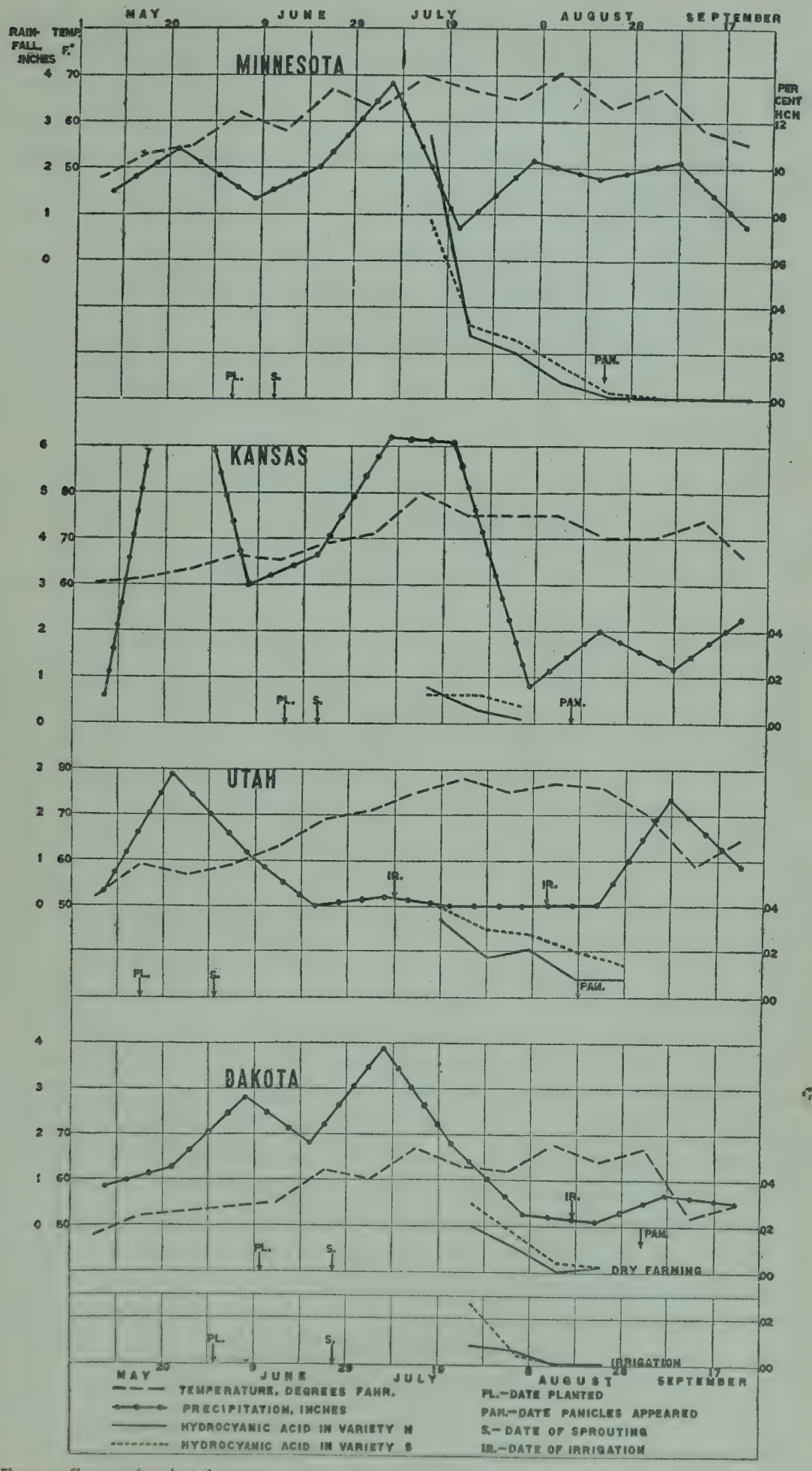


FIG. 4.—Curves showing the contemporary climatic conditions at the various plots, together with crop data and hydrocyanic-acid content.

Accompanying these various conditions were also widely differing amounts of hydrocyanic-acid glucosid. How the correlations between these two may be explained will depend upon the function assigned to the dhurrin in sorghum. Various uses have been attributed to glucosids in plants, as (*a*) a protection against bacteria and other enemies by means of the poison set free when some glucosids are hydrolyzed; (*b*) a reserve food material in the plant; (*c*) the inactive form of a stimulating hormone (*z*) set free when necessary by a glucosidase; (*d*) a harmless compound absorbing injurious products of metabolism; (*e*) an inactive storage of "respiratory pigments," and other uses.[1] Hydrocyanic acid itself is thought by some investigators to be a necessary intermediate product in protein formation (6, 8). As such, it is probably rather transitory in the plant, and seldom occurs free in any appreciable amount.

A discussion of each factor which might have any bearing on the cause of the variations in cyanid content, or throw any light on the function of dhurrin in sorghum, follows.

1. HUMIDITY.—It is hard to perceive how the relative humidity might have any direct bearing on the quantity of dhurrin produced. The humidity affects primarily the rate of transpiration, and this in turn might influence the rate of growth. The latter factor is considered in the next paragraph. The interesting thing to note in the humidity curves in figure 2 is the fact that the Utah curve shows a decrease during a period of decreasing precipitation, which is natural, but the Kansas and Minnesota curves show an increase during periods of decreasing precipitation. It is possible that this very low humidity in Utah caused a rate of transpiration too high for the best development of the plants, and their growth was retarded accordingly. When the humidity was lowest, in July and August, the plots received their two irrigations. Following these the growth was more rapid. If the humidity affects the amount of glucosid at all, it is by means of its effect on the nutrition and growth of the plant.

2. MOISTURE SUPPLY.—As mentioned above, there are among the four stations one having very high, one with very low, and two with medium rainfall. Two plots at the last-named stations were under irrigation and one under dry-farming methods of cultivation. In the data as a whole there is no evident correlation between the amount of the glucosid and the moisture supply for the five months. Arranging the stations in the order of their moisture supply, they are Kansas, Minnesota, South Dakota irrigation, South Dakota dry farming, and Utah; while arranged in the order of their cyanid content they are Minnesota, Utah, Kansas, and South Dakota dry farming the same, and South Dakota irrigation. However, by a closer examination of the curves for

[1] For a complete discussion of the function of glucosids in plants see Armstrong, E. F., The Simple Carbohydrates and the Glucosids. Ed. 2, p. 125–133. London, New York, 1912.

each plot, the following examples tending to show that high water supply is often accompanied by a low cyanid content are discernible: (1) The normal hydrocyanic-acid curve for sorghum during the first two-thirds of its growth is a smooth curve, with a steady decrease in the acid. The Utah curve is an exception to this. In this plot there was for several weeks very rapid transpiration of water, owing to low humidity; hence, the plants and soil were reduced nearly to the minimum water requirement. Shortly after the first irrigation the hydrocyanic acid is seen to be on a normal decline. Twenty days after this irrigation, however, the plots had become comparatively dry again, and the hydrocyanic acid shows a less decrease in variety S and an actual increase in variety N. The second irrigation was followed by another decline in hydrocyanic acid. By the latter part of August the need of water was once more felt, and the cyanid in variety N, at least, had ceased to decrease. (2) The curve for the South Dakota dry-farming plot also shows an abnormality in that the last part of it has an upward turn in the case of variety N. It is possible that this may be due to the smaller supply of moisture available at this time. (3) In the two South Dakota plots, both received the same amount of rain; one was irrigated once and the other, being cultivated by dry-farming methods, had a larger reserve supply of water. This would apparently give them about the same amount of water supply, except for the fact that the irrigation was a heavy one, and the heavy rains during May, June, and July disturbed the usual dry-farming condition of the soil. Assuming that the irrigated plots did have more water available, it will be seen that they also contained a less amount of hydrocyanic acid. (4) On analyzing some sorghum plants grown in pots in the greenhouse, they were found to contain no hydrocyanic acid. A few weeks later some larger plants from this same group, growing in drier soil, owing to lack of care in watering and to a larger demand made by the plants, were found to contain some of the acid. There appears, therefore, to be a relation between the supply of water and the amount of dhurrin present. This may be explained on the hormone theory With a liberal supply of water, other things being equal, the plant's means for growth are adequate and it needs less glucosid. With a decreasing water supply, however, the plant may need the hormone stimulus for growth, and more glucosid is produced. Although, as shown by Briggs and Shantz (5), sorghum has a lower water requirement than most cultivated plants, it is no doubt affected by changes in the supply of moisture.

3. Temperature.—No correlation has been found between the content of dhurrin in sorghum and variations in temperature, at least for the range of temperatures which obtained during this experiment. The increase in hydrocyanic acid which sometimes occurs when plants are frosted may be due to disturbed enzym balance.

4. RATE OF GROWTH.—The Kansas and Utah plots present extremes in rate of growth and thriftiness of the sorghum plants; and they also present cases of relatively low and high hydrocyanic-acid content, respectively. The cane on the Minnesota plots grew more slowly than that from South Dakota, and it also contains a very much higher hydrocyanic-acid content. During the first four or five weeks on the Minnesota plots the plants grew very poorly, the weather being cold and damp. The plants were yellow and uneven in height, similar to those obtained from Utah. The samples from these two stations were by far the highest in hydrocyanic acid. In fact, the percentage in the first Minnesota sample, variety N (0.114 per cent), is the highest ever observed in the authors' experience with sorghum. In the Minnesota samples of 1914 those grown on the poorer sandy soil were the higher in cyanid. These examples, together with one furnished by Avery (3), show that some significant relation may exist between poor conditions of growth and high dhurrin content. In opposition to this, however, is the finding of Alway and Trumbull (1) that the yellower plants in a field contained a smaller amount of the acid. Balfour (4) found more in plants infested with *Aphis sorghi* than in others not so affected. If these facts are now applied to the various theories mentioned above, as to the function of glucosids, some of the possibilities are as follows: (1) If this particular glucosid is a food storage, it is difficult to see how it could exist in largest quantities in the unhealthy, poorly nourished, slow-growing plants. (2) If the constituents of the glucosid act as stimulating hormones when set free by an enzym, it is possible that when conditions of growth are poor more of the glucosid is produced. (3) If the glucosid is an absorber of harmful products of metabolism under disturbed metabolic conditions, an excess of hydrocyanic acid might be produced. Of these three the authors believe the second to be the most tenable for dhurrin, according to the available evidence on this question.

5. VARIETY.—The most striking phenomenon in this experiment is the fact that Variety S has consistently a greater amount of hydrocyanic acid than Variety N. That varietal difference is very important was brought out also in the 1914 experiments. In fact, the authors are confident that the most marked and constant differences in the hydrocyanic-acid content of various sorghum plants will be found to be due to variety rather than to external conditions. A comparative study of the glucosid content of all varieties of sorghum would be interesting and valuable.

6. DISTRIBUTION IN THE PLANT.—The foregoing discussion has been based on the dhurrin content of the whole plant. As is seen from Table II, the distribution of the glucosid between stalk and leaves in the different plots is variable. There is in every instance a more rapid decrease in the stalks than in the leaves, but the comparative rate of decrease varies. The Minnesota and Utah plots had the highest amount in the stalks and also had the slowest growth and the thinnest stalks. The Kansas and

the South Dakota plots, on the other hand, had little or no cyanid in the stalks and had the most rapid growth. The Kansas stalks were very heavy and succulent; they had developed very rapidly; and they contained no cyanid whatever. The significance of this is not clear.

7. DAILY VARIATION.—In order to compare the glucosid content in sorghum with Treub's findings (7, 8) that in *Pangium edule* there is a daily variation in glucosid content with a maximum about midday, some analyses were made at sunset and sunrise of succeeding days, with the results given in Table III.

TABLE III.—*Variation in the glucosid content of sorghum at different parts of the day*

| Variety. | Part of plant. | Percentage of hydrocyanic acid. | |
|---|---|---|---|
| | | Evening. | Morning. |
| Variety N | Leaves | 0.015 | 0.012 |
| Do | Stalks | .013 | .014 |
| Dakota Amber | Leaves | .020 | .023 |
| Do | Stalks | .023 | .031 |

There seems to be no constant variation in sorghum between night and day. This lends support to the view that dhurrin is not a food storage.

Although other factors have important bearing on the growth and health of plants, those discussed above are the most readily measured and, hence, best used as bases for comparison between widely separated stations. It is realized that determinations of soil moisture at various times throughout the growing season would give a much more accurate idea of the available moisture than precipitation measurements. As regards soil, each plot was grown on soil which has produced good crops in the past and was cultivated according to the customary methods for sorghum at those stations. Since the 1914 experiments showed that soil is a minor factor in affecting the hydrocyanic-acid content of sorghum, the ignoring of this factor in the above comparisons is justified.

## SUMMARY

Two varieties of sorghum, Southern Cane and Early Amber, were grown on plots in Minnesota, Utah, Kansas, and South Dakota under widely different climatic and cultural conditions. The amount of the glucosid dhurrin in each plot varied considerably. The following correlations relative to the amount of glucosid were found to exist.

(1) Unhealthy plants usually contain more hydrocyanic acid than healthy ones. The unhealthy condition may be due to malnutrition, to improper transpiration, to insect attack, or to other causes. It is possible that under such conditions the plant produces more glucosid for the sake of the stimulating hormones in it.

(2) The apparent effect of humidity and temperature on the amount of cyanid in sorghum is probably due to the indirect effect on the rate of growth.

(3) Adequate water supply is usually accompanied by low, and inadequate by high, hydrocyanic-acid content. This is probably due to the need of glucosid stimulation when the water supply becomes low.

(4) The character of the growth of the plant affects the distribution of dhurrin between leaves and stalks, there being a proportionately smaller amount in the thick, heavy stalks than in the slender ones.

(5) There is no consistent daily variation in the amount of dhurrin, which argues against the functioning of this glucosid as a food storage.

(6) Of the two varieties used in this experiment, the Southern Cane in every plot but one had a higher content of hydrocyanic acid than the Early Amber. Varietal difference is probably of more weight in determining the amount of hydrocyanic acid in sorghum than are the conditions of growth.

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# EGG AND MANNER OF OVIPOSITION OF LYCTUS PLANICOLLIS[1]

By Thomas E. Snyder,
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## HISTORICAL SUMMARY

The so-called "powder-post" injury to seasoned wood products is widely distributed over the world. Of the various beetles causing this type of injury, species of the genus Lyctus Fab. are by far the most important. While these beetles and their damage have an extensive literature, the place and manner of oviposition have remained obscure. Heeger (3),[2] in 1853, described and figured the egg, larva, and pupa of a beetle attributed to a European species, *Lyctus pubescens* Panzer. Dugès (1),[3] in 1883, described and figured the larva, pupa, and adult of *L. planicollis* Le Conte (?), proving that Heeger was in error in ascribing the larva he figured to the genus Lyctus. Xambeu (7), in 1898, described the egg and manner of oviposition of *L. linearis* Goeze (*canaliculatus* Fab.). Recently the eggs of the native species, *L. planicollis* of the southern United States, have been found by the writer. This egg is very unlike that described and figured by Heeger as the egg of *L. pubescens*, and it differs from the egg of *L. canaliculatus* as described by Xambeu, being of a most unusual type for Coleoptera.

The following brief notes on the mating and oviposition of the southern species (*L. planicollis* Le Conte) were made on material being reared either at Washington, D. C., or at Falls Church, Va., in buildings kept dry and at a temperature above freezing.

## LIFE CYCLE

### MATING

The beetle passes the winter in the larval stage, but in cold weather the larvæ are more or less dormant and infested stock may consequently pass unnoticed. Mating takes place and the eggs are deposited soon after the adult beetles emerge from the wood in the spring. At Washington, D. C., and Falls Church, Va., the first adults emerged from infested wood in rearing cages during the last part of February and first part of March,

[1] The specimens on which this paper is based were identified by Mr. W. S. Fisher, Specialist on Forest Coleoptera, of the Branch of Forest Insect Investigations, Bureau of Entomology.

[2] Reference is made by number to "Literature cited," p. 276.

[3] According to Dugès, the material on which his paper was based had been determined by two different authorities as *Lyctus planicollis* Le Conte (of southern U. S.) and *carbonarius* Waltl. (of Mexico and Florida). Dugès refers to the species as *planicollis* in the title and *carbonarius* on the plate. Hopkins (5, p. 134) states that *L. carbonarius* is evidently distinct from *L. planicollis*, and therefore Dugès's specimens are *L. carbonarius*.

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in 1914 and 1915. At Baltimore, Md., adults of this species emerged from an infested oak table in a heated building as early as January 12, 1916. General emergence at Falls Church, Va., however, did not begin until about the middle of April, 1914 and 1915. The period of maximum activity is from the last of April to the first part of June. The last adults emerged during the first part of July. Mating occurred commonly during May, in 1915.

## OVIPOSITION

Oviposition began a few days after mating and was observed to take place principally during the middle of May, in 1915. On May 24, 1915, many beetles were observed on radial sections of wood with their ovipositors deeply inserted into the open ends of pores or large longitudinal vessels in the wood, but the first eggs were not found till June 1, 1915.

The beetles seem to prefer to oviposit on those sections of seasoned sapwood where the open ends of pores are most numerous. These pores are especially prominent in "ring-porous" woods such as hickory, ash, and oak, which are also the species most subject to attack by Lyctus beetles. No eggs were observed on the surface of the wood, but all that were found were in these pores.

The females remain for several minutes with the ovipositor in the pore, and the process is repeated at several places. The female usually assumes a position in which the body is parallel to the pore and the ovipositor is either curved down and bent forward into the pore underneath the body or projected directly into the open end of the pore. However, the ovipositor, which is long and flexible and reaches from the end of the body to the thorax when extended forward, can be projected in any direction. At the extremity of the ovipositor are two laterally placed palpi. In the process of inserting the ovipositor into the pores, there is a considerable preliminary period of thorough examination with these palpi of all parts of the pore before an egg is laid. Two or more eggs are usually laid near together in each pore utilized. Each female deposits eggs in several pores.

## THE EGG

The egg (Pl. XXVIII, fig. 1) is cylindrical, rounded at the ends, and has a slender strand or process attached to the cephalic pole. It is whitish in color, somewhat shiny, 1 mm. in length with the strand attachment, 0.75 mm. in length without this process, and 0.175 mm. in width. This process or strand is somewhat similar to that of the eggs of certain parasitic Hymenoptera—that is, parasites of the cotton boll weevil in the families Eurytomidae and Encyrtidae (6, p. 49–51, pl. 2), but this is the only instance known to the writer of such a process on the eggs of Coleoptera. The egg has a granular appearance (Pl. XXVIII, fig. 2), and at the end which terminates in the process there is an area marked with parallel, longitudinal striæ (Pl. XXVIII, fig. 4). The egg of *L. linearis*

(*canaliculatus*), as described by Xambeu, is very different from the egg of *L. planicollis*, since no mention is made by Xambeu of either the strand attachment or the area of longitudinal striæ, which are unusual characters in the egg of a beetle.

The end with the process (the cephalic pole) leaves the ovipositor last,[1] and this strand may possibly be attached by the ovipositor to the pore contents. The larva does not occupy much more than half the length of the egg (Pl. XXVIII, fig. 3). In hatching, the larva backs out of the egg. The eggs are easily broken, and it is probably due to this fragility and the fact that they are inserted far into the pores that the eggs of Lyctus beetles have apparently not been previously observed with absolute certainty of their identity.

## SEASONAL HISTORY

Egg laying takes place principally during the middle of May. Recently hatched larvæ were first observed on June 1, 1915. The period of incubation is probably, at most, 10 days. The winter is passed in the larval stage. General pupation occurs about the first of April; the pupal cell (Pl. XXX) is excavated near the surface of the wood, and to this cell the larvæ retreat after cutting a transverse burrow nearly to the surface for the exit of the adults. General emergence of the adults takes place during May. Under normal conditions of the natural habitat of this species (in the Gulf and South Atlantic States) activity probably occurs earlier in the season.

There is apparently only one generation annually. But the combined work of the many larvæ of successive broods and generations burrowing through the wood results in the complete destruction of the interior and the conversion of the wood into fine powder—that is, "powder-posted" wood (Pl. XXIX, XXX, and XXXI).

## CONCLUSIONS

Injury by "powder-post" beetles to unfinished seasoned wood products can be prevented by simply adapting a system of inspection, classification, and methods of disposal of stock to facts in the seasonal history of the insects, as has been recommended for many years by Hopkins (4, p. 6), Forest Entomologist. Such methods have been adopted by several large manufacturing companies with marked success.

In the case of finished wood products it may often be practicable to treat the wood with substances to prevent attack. Creosotes are effective preventives, but they stain the wood; hence, where they can not be used, in the light of the discovery of the place and manner of the laying of the eggs, any substance that will close the pores will prevent oviposition in wood not previously infested. In wood from which beetles have

[1] This is according to the law of orientation of Hallez (2).

emerged, however, eggs might be laid within the exit holes. Paraffin wax, varnish, or linseed oil effectively closes the pores of wood. Wood that has been seasoned less than 8 to 10 months will not be attacked by Lyctus beetles. In applying chemical preventives, only sapwood that has been seasoned for 8 to 10 months and longer should be treated. Judging from facts in the seasonal history of this species, preventives should be applied before March 1.

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## PLATE XXVIII

*Lyctus planicollis:*

Fig. 1.—Outline of the egg, showing strand attachment.

Fig. 2.—Greatly enlarged view of end of egg, showing granular appearance.

Fig. 3.—Larva within egg, ready to hatch. Drawn by Miss M. Carmody.

Fig. 4.—Sketch of egg in pore of wood on radial section of green-ash (*Fraxinus lanceolata*) ladder-rung stock, showing longitudinal striæ; pore opened to show egg. Drawn by C. T. Greene.

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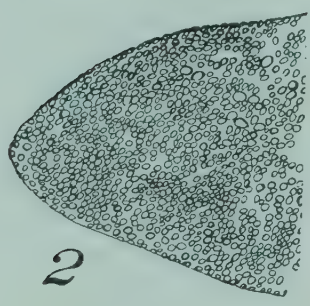
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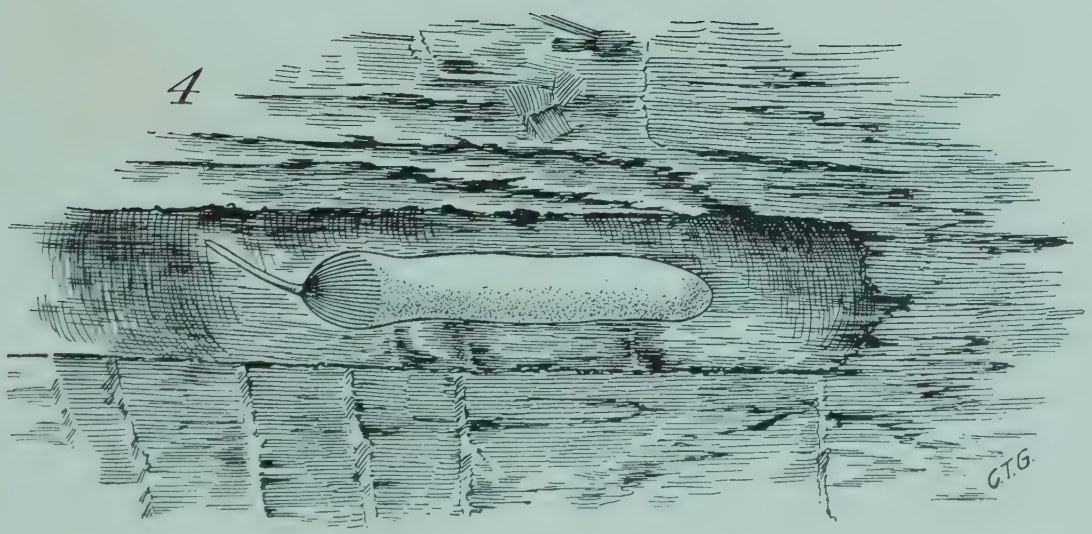
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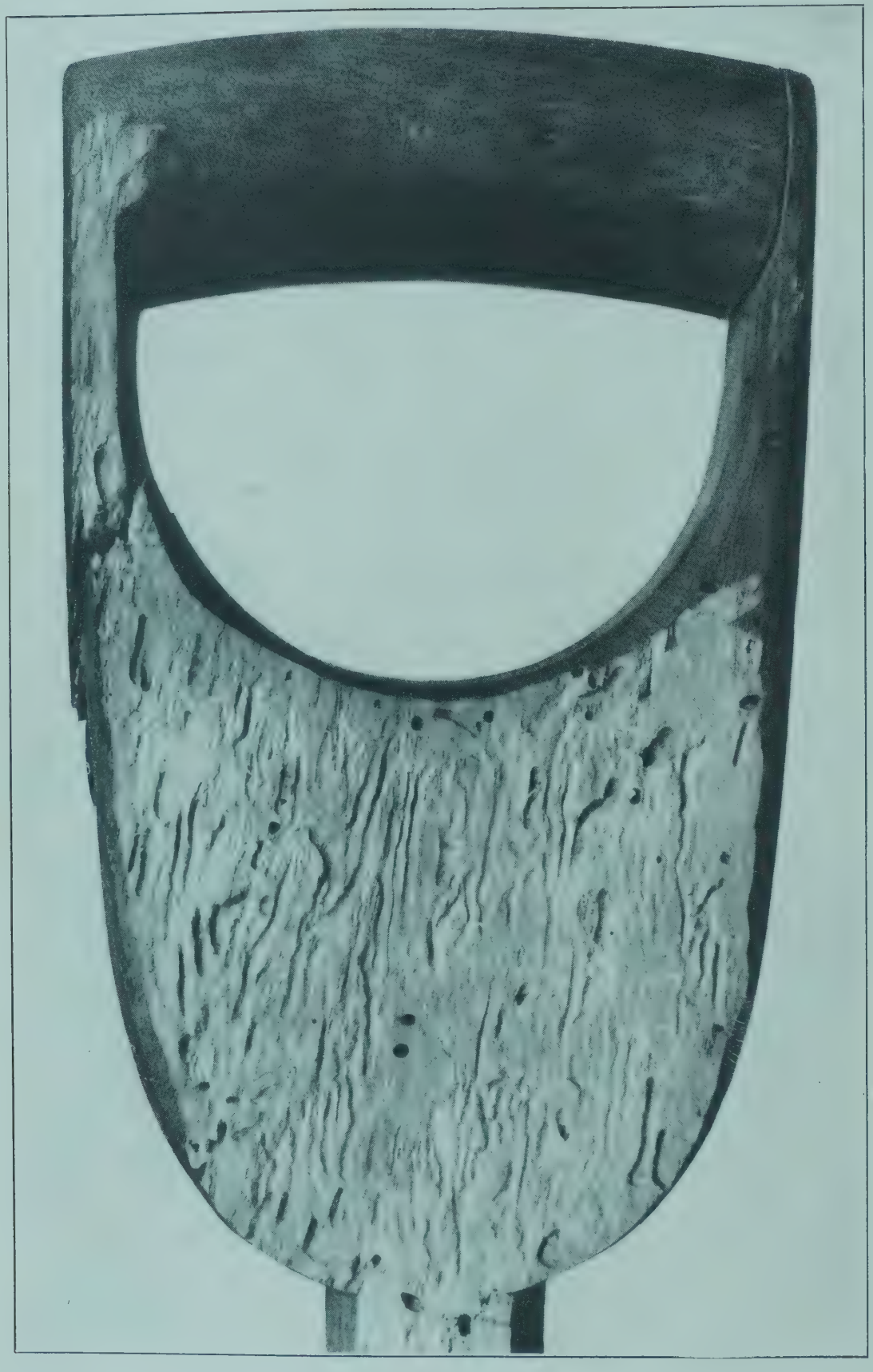


## PLATE XXIX

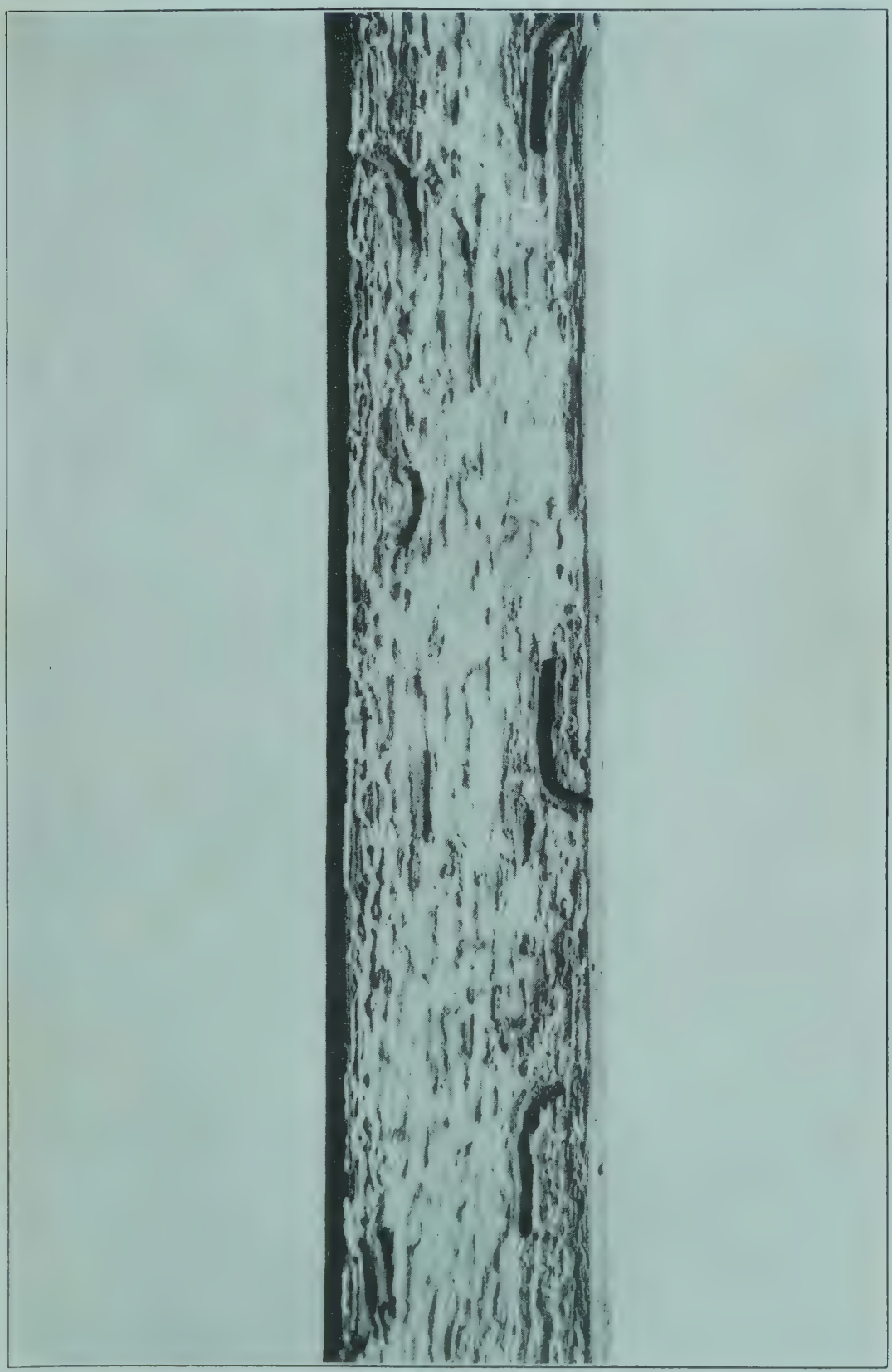
*Lyctus planicollis:*

Larval burrows in an ash shovel handle. Handle planed to show the work of the larvæ. Photographed by H. B. Kirk.

## PLATE XXX

*Lyctus planicollis:*

Pupal cells in "powder-posted" white-ash shovel handle. Photographed by H. B. Kirk.



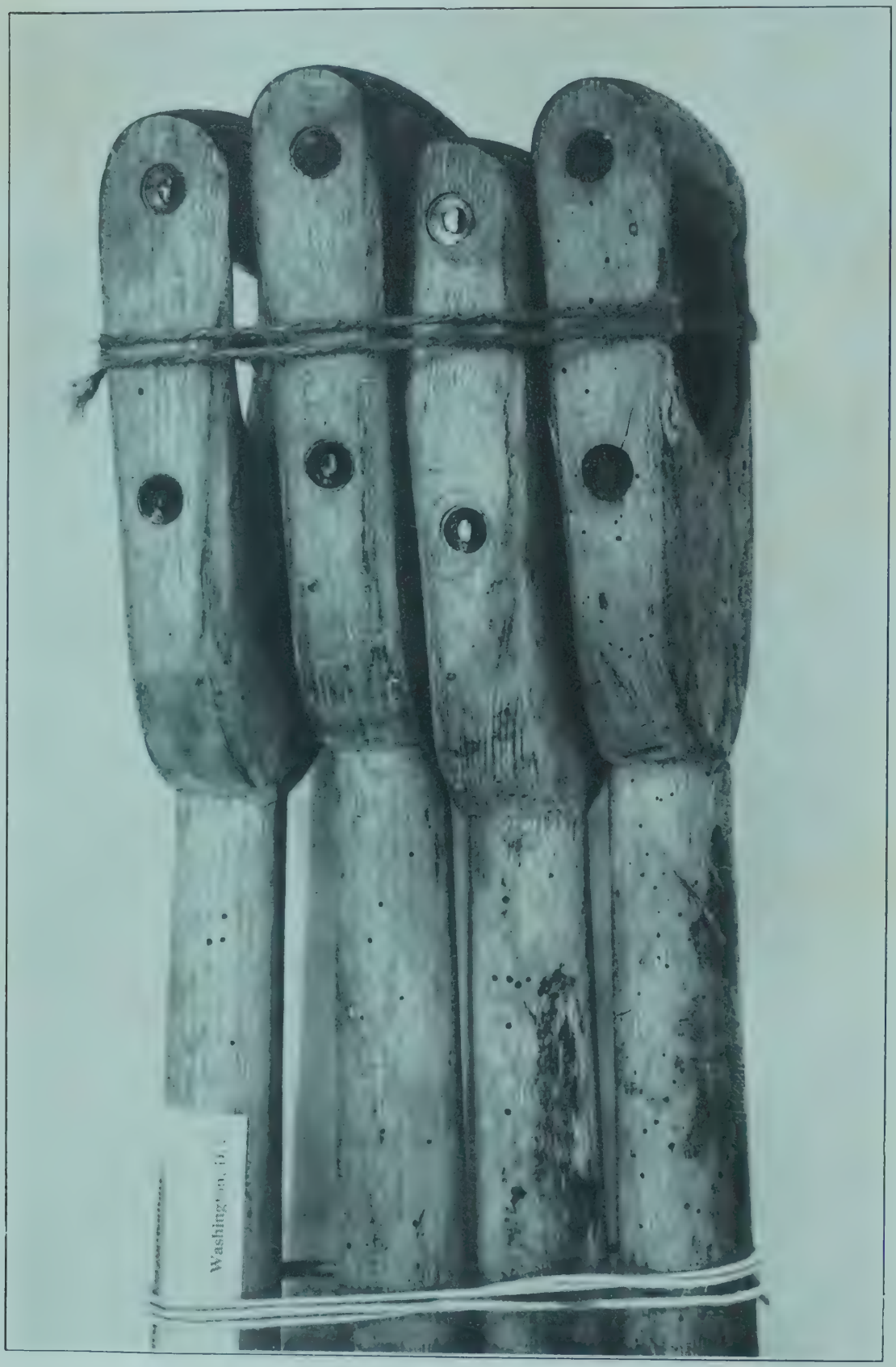


PLATE XXXI

*Lyctus planicollis:*

Exit holes of adults in ash shovel handles. Photographed by H. B. Kirk.

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# HYPODERMA DEFORMANS, AN UNDESCRIBED NEEDLE FUNGUS OF THE WESTERN YELLOW PINE

By JAMES R. WEIR,
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## INTRODUCTION

In the summer of 1913 the writer's attention was drawn to what appeared to be a very serious needle disease of the western yellow pine (*Pinus ponderosa* Laws.) in parts of Idaho, Washington, and Montana. That the disease has become more prevalent is shown by the receipt at the Laboratory of Forest Pathology at Missoula, Mont., of many collections of the fungus from localities where it was not before known to exist. These collections represent material from trees of all ages and show the youngest needles as badly diseased as the oldest ones. The first suspicion that the fungus might be of some economic importance arose through the discovery of a serious infection of young reproduction over a large area in the Whitman National Forest, Oregon. From the fact that the fungus causes a conspicuous hypertrophy by the extension of its mycelium into the tissues of the twigs and also through the destruction of the youngest needles, consequently causing in some localities much damage in the forest, it seems desirable to make known its characteristics.

## TECHNICAL DESCRIPTION OF THE FUNGUS

Since the fungus does not agree with any known member of its genus, it is described as new.

**Hypoderma deformans**, n. sp.

Apothecia black, shiny, averaging 10 mm. in length and 1 mm. in breadth; may extend as a black line the entire length of the sheath side of the needle or be broken up into a series of shorter apothecia, usually arranged along the middle line of the needle, but may appear at either side and be very rarely confluent with the more medially arranged apothecia; opening with a longitudinal medial split. Asci fusiform (26) 26.1 to 43.5$\mu$ by 159.5 to 207.2$\mu$ (27.3 to 29.0$\mu$ by 171.5 to 186.4$\mu$). Spores parallel or obliquely arranged in the ascus, very generally slightly curved, uniform breadth, rod-shaped, ends blunt, 1-septate when mature, septum very conspicuous, cells often apparently separated, pale olive, almost hyalin, eight to an ascus (40) 6.2 to

9.7μ by 90.67 to 131.37μ (7.4 to 8.7μ by 108.9 to 117.6μ); paraphyses numerous, filamentous, swollen at the ends or recurved. Spermogonia intermixed averaging 5 mm. in length; spermatia elongated, straight, sometimes slightly curved, hyalin, continuous, averaging 1 by 8μ.

Type locality: Sumpter, Oreg., Whitman National Forest.

Habitat: Living needles of *Pinus ponderosa*.

Type material deposited in the Office of Investigations in Forest Pathology, Bureau of Plant Industry, Washington, D. C., and in the collections for study in the Laboratory of Forest Pathology in the same office, at Missoula, Mont.

## GENERAL BIOLOGY OF THE FUNGUS

The apothecia of the fungus are the most conspicuous of any of the group on pines in the West (fig. 1). From new infections of the previous year fully mature apothecia with well-developed spores (fig. 2) may be collected in early spring. From this time on the longitudinal split on the medial line of the apothecium is plainly visible, and may remain open or closed, depending on the humidity of the atmosphere.

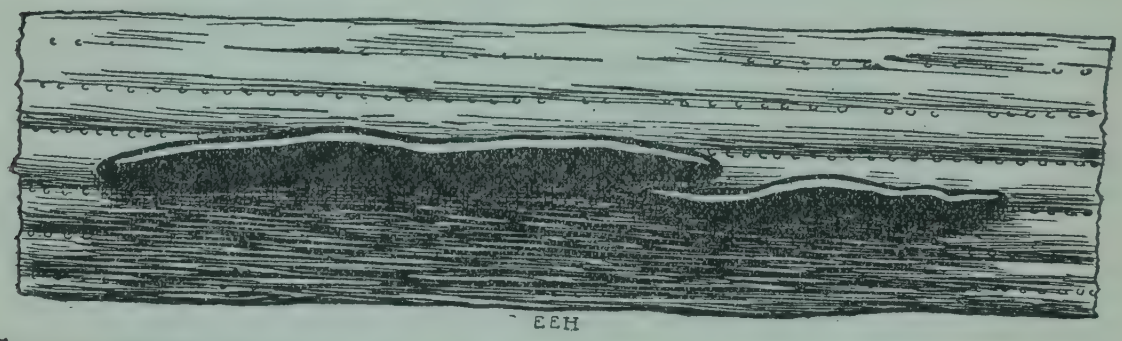


FIG. 1.—A side view of two apothecia of *Hypoderma deformans* on needles of *Pinus ponderosa*, showing the longitudinal medial split.

The splitting of the epidermis on the needle directly on the medial line of the apothecium is a characteristic shown by nearly all of the Hysteriaceae and in a few cases seems to be governed by a particular structure of the overlying layers of the apothecium. Thus, Von Tubeuf [1] points out that the pseudoparenchymous covering of the apothecium of *Lophodermium pinastri* (Schrad.) instead of being one continuous homogeneous tissue is made up of two parts which come together on the middle line of the fruiting body. The edges of the two parts interlock by a series of short papillæ. It is on the line of these papillæ, when the pressure within the apothecium becomes sufficient, that the epidermis of the needle ruptures. In *Hypoderma deformans* the rupture of the apothecium is apparently made easier by the coalescence of filamentous elements springing from the floor of the apothecium and meeting with the darker tissues of the apothecial covering above. Owing to a differentiation of the covering of the apothecium at the point of union a line of rupture is formed.

[1] Tubeuf, Carl von. Studien über die Schüttekrankheit der Kiefer. *In* Arb. Biol. Abt. Land- u. Forstw. K. Gsndhtsamt., Bd. 2, Heft 1, p. 22, 1901.

Pressure within the apothecium on approaching maturity, together with the elongation of the central elements, causes the rupture to occur on this line. After initiating the line of rupture, the filaments disappear and no sign of their presence exists when the spores are mature. In all material so far examined this mechanism is a constant characteristic. Where two apothecia are formed side by side, the filamentous structures are in marked contrast to the division line between the two apothecia as formed by the union of the darker colored elements of the apothecial covering. Von Tubeuf found in *Hypoderma strobicola* Tub. (*Lophodermium brachysporum* Rostr.) the same structure which he describes for *Lophodermium pinastri* (Schrad.), but no such structures were found in *H. deformans*.

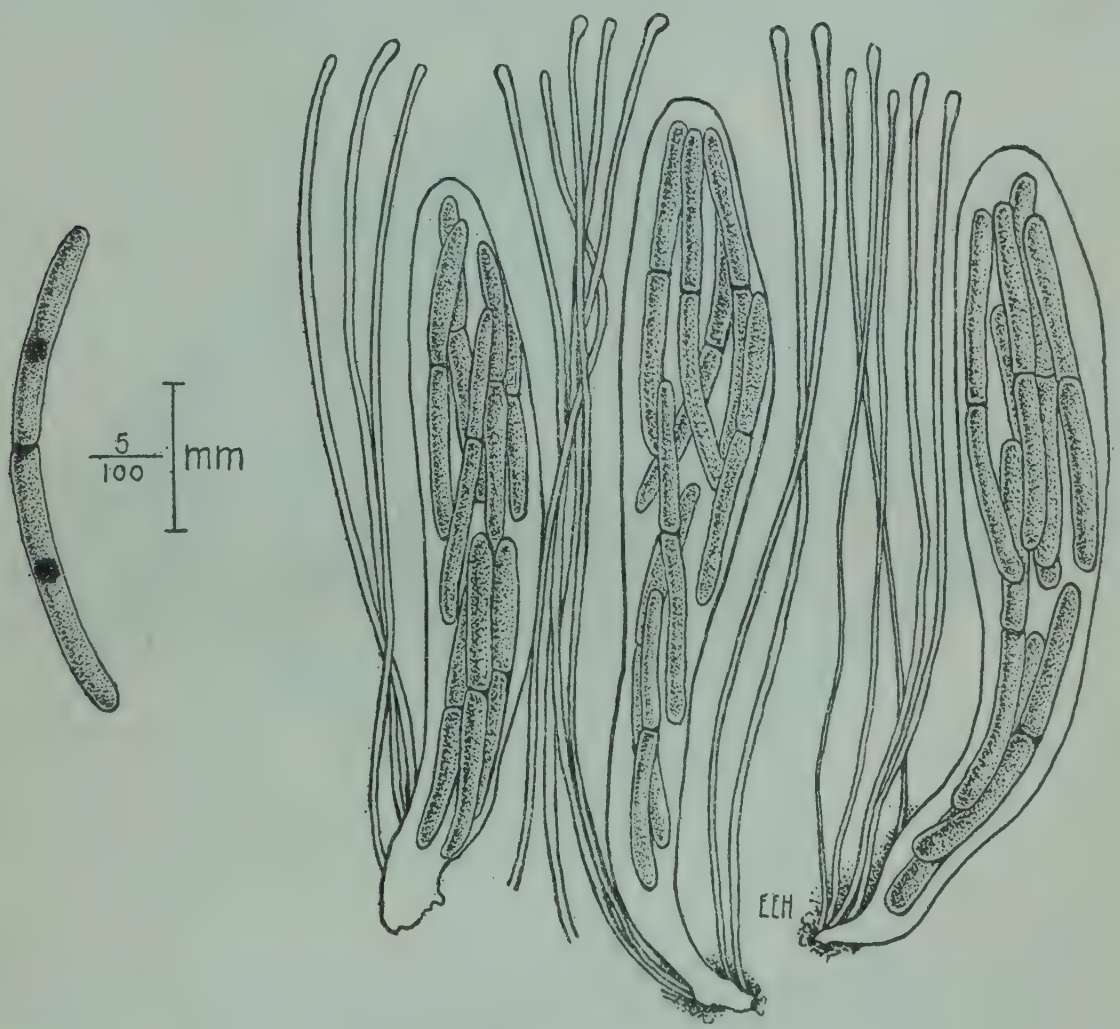



FIG. 2.—Asci, spores, and paraphyses of *Hypoderma deformans*.

Apothecia with mature spores (fig. 3) may be found at any season of the year. This is due to the fact that the spores do not ripen or are not all freed simultaneously when the split first appears in the apothecium. The process of spore liberation is observed to extend over a long period of time. A year may elapse before the apothecia have entirely liberated their spores. During periods of drought the medial slit in the apothecial covering remains closed, only opening on the return of abundant

moisture. The hygroscopic movements of the lips of the apothecium furnish the method by which the spores are forced or ejected from the asci. As Von Tubeuf [1] has pointed out in the case of *Lophodermium pinastri*, the spores are shot out from the mature asci under proper conditions of moisture. This fact is easily demonstrated by inclosing short pieces of previously moistened needles bearing mature apothecia in the cavities of plate-glass culture slides. A microscopic study of such preparations shows that the spores are shot out from the asci a distance of from 1 to 2 mm., showing as a plainly visible deposit on the floor and cover of the cavity. The depth of the cavity in the slides used was 2 mm.

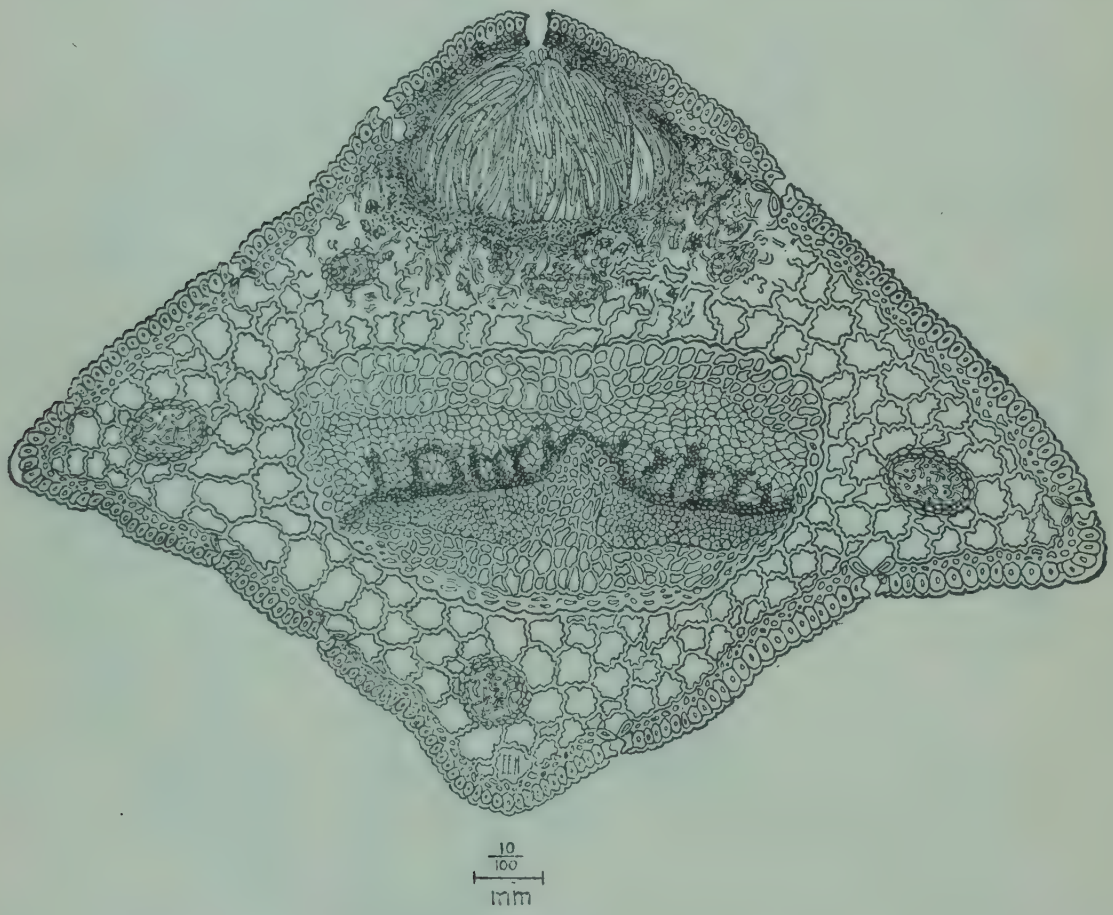



FIG. 3.—Cross section of an apothecium of *Hypoderma deformans* on a needle of *Pinus ponderosa*, showing mature asci with spores, the point of first rupture, and the tissues of the leaf most seriously affected by the mycelium of the fungus.

Occasionally an entire ascus was ejected and lay among the spores. In most cases, the asci remained attached and the spores were expelled through their terminal pores (fig. 4). Only the fully developed spores were cast out of the apothecia. After the material had remained in the slides a day and a half, during which time the spores were being ejected, the cover glass of a slide was removed and the material allowed to dry by exposure to the air of the laboratory for 30 days. The material was washed and replaced in the cavity in the slide. Within three hours spores from the same apothecia were expelled in considerable numbers but not so profusely as before. The process was repeated with shorter

[1] Tubeuf, Carl von. Op. cit., p. 24–25.

periods of drying till on the fourth trial no spores were liberated. An examination of the apothecia showed the asci to be entirely empty. This experiment not only demonstrates that the fungus has the ability to resist protracted periods of dryness but that the period of spore liberation may be much protracted, depending upon the atmospheric humidity. During wet weather apothecia expel their spores in visible quantity when a sharp blow is given the branch bearing infected needles.

Considering the long periods of drought in most yellow-pine regions, it is safe to assume that an apothecium ripening in early spring may first become emptied of its spores during the ensuing winter or even later. This is important for the propagation of the fungus, since new infections are possible from the time the first needles of the season appear till the close of the growing season.

In order to determine the viability of the spores expelled from apothecia after long dryness, a 2 per cent sugar solution was introduced into the cavity of one of the slides containing apothecia which had lain dry in the laboratory for two months and the slide placed in the thermostat at 35° C. On the fourth day spores germinated readily. The germ tubes appeared more frequently from the ends of the spores. A slight addition of an extract of pine needles to the sugar solution promoted germination.

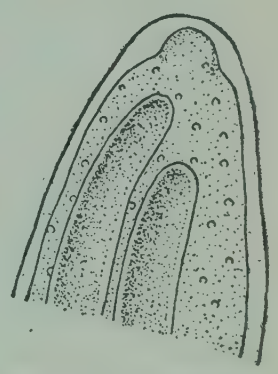


FIG. 4.—The upper portion of a young ascus of *Hypoderma deformans*, showing the formation of the pore at the tip through which the spores are expelled.

It was noticed that in collections of the fungus made shortly after warm summer rains the asci are frequently empty as compared with asci of mature apothecia collected in the colder spring months. This, it seems, may not be entirely due to a longer period of spore liberation but also to the higher temperature of the summer months. Von Tubeuf found that increasing temperature promoted spore liberation in *Lophodermium pinastri* and it is found to be true in experiments with the yellow-pine fungus. During the winter, moistened apothecia from dry material were mounted in two culture slides; one was placed outside the laboratory during a period when the thermometer registered about 40° F. and the other was kept in the laboratory air of about 80° F. At the end of four hours a microscopical examination showed that a large number of spores had been ejected from the apothecia in the slide kept in the laboratory but none from the other slide. When the slide from the outside was allowed to stand for a while in the warm air of the laboratory, spores were liberated in quantity.

Although spores from various needle fungi are undoubtedly more readily liberated during warm rains of the summer months, the frequent drying of the foliage of the trees is probably not favorable for infection. It is frequently observed, and as often reported, that needle fungi become more active during the cool, protracted rainy periods of early spring and late fall. No extensive data are at hand regarding the resistance of

expelled spores to drought and direct light; still, the fact that dry herbarium material a year old was found to furnish viable spores shows that spores may exhibit considerable resistance to dry air when free from the apothecium.

## PARASITISM OF HYPODERMA DEFORMANS

An attempt to grow the fungus on culture media failed. The spores in every case germinated and in some cases produced an abundant white mycelium, but in the course of six months, after frequent transfers, the mycelium turned a light yellow and died. A somewhat better result was obtained by adding to the culture medium a strong extract made from yellow-pine needles in water, but at the end of eight months the mycelium died.

A quantity of needles bearing apothecia with mature spores were collected in the spring of 1914 near Missoula, Mont., and taken to the field station in the Priest River Valley, Idaho, for experiments on parasitism. The fungus has not been found in this region. The needles were thoroughly washed in distilled water and the apothecia allowed to expel their spores in small sterilized flasks. Needles and spores were shaken up in water to which a 1 per cent sugar solution was added. The mixture was allowed to stand one day and then thoroughly sprayed over four 3-year-old yellow-pine seedlings having young tender shoots with needles. The inoculated seedlings were immediately inclosed in tough, transparent oiled paper bags and protected from injury. A second experiment was initiated by binding infected needles on healthy 3-year-old seedlings. In the part of the Priest River Valley where these experiments were performed the yellow pine is not common, being only sparingly represented in a mixture of white pine, grand fir, spruce, hemlock, and Douglas fir. The experiments were made on May 20. In September the last-formed needles of the inoculated seedlings were turning reddish brown in spots, mostly at the tips. In the following spring, May to June, the needles which showed infection in the fall and which had become wholly brown developed the characteristic long, shiny black apothecia with mature spores (Pl. XXXII, fig. 1). Only the needles formed during the previous year were infected. Four control plants, also covered with bags, were entirely free from the disease. The needles of the seedlings on which infected needles were bound showed a much more general infection of the last-formed needles than those by the former described method. In these experiments every needle produced in 1914 was infected. Those of previous years remained healthy. This indicates that old needles are not attacked and that the young needles may remain attacked indefinitely after infection. All the infected needles did not produce mature apothecia. Those merely turning brown were filled with the mycelium of the fungus. The experiment at this point was discontinued. In all probability, given time enough, the brown-infected needles would have produced apothecia.

It has been noticed repeatedly in nature that there is great irregularity in the time between the first browning of the needles at their tips or at other points along the needles and the appearance of the mature apothecia. In a few cases the cycle of development from the first appearance of the brown color at the tips of the needles to mature apothecia has been observed to take place within the same calendar year, or from April and May to November. More often infected needles first showed mature spores in the spring of the following year. It was observed in a few cases that the needles may lie on the ground through the following winter before the apothecia rupture. Brown needles collected in August from infected trees and placed in damp moss in the field in a number of cases developed apothecia before January, maturing in May and June. The apothecia, as previously indicated, may contain asci in various stages of development, so that mature spores are being produced throughout the year. Investigation has shown, however, the greatest number of spores are expelled during the spring rainy season, May and early June, coinciding with the greatest vegetative period of the host. In no instance, either in the field or in artificial inoculation, were the infected needles of young trees or seedlings not previously attacked by the fungus killed before they had attained their normal size. In September or October, such needles will have assumed a more or less uniform reddish brown color. Mostly remaining upon the tree, they may first produce the signs of the apothecia during the late fall and mature the spores in the following spring. At the time the foregoing experiments were in progress small bundles of infected needles bearing fertile apothecia were bound with similar quantities of needles which had died from a normal cause. These were placed in moss during May, 1914. On examination in May of the following year the needles which had died from a normal cause showed no signs of the fungus; nor have they done so since that date. This apparently demonstrates the inability of the fungus to act as a saprophyte.

The foregoing observations and experiments apparently prove the parasitism of the fungus. This is further substantiated by the observed evidences that young seedlings in the field succumb to the ravages of the fungus. Furthermore, it is indicated that the period of greatest infection is during the growing season and only the needles of the season are to any extent susceptible to attack.

The fungus has not yet appeared in the forest nursery, but it may be regarded as a possible nursery disease.

## PATHOLOGICAL EFFECTS OF THE FUNGUS ON THE BRANCHES OF THE HOST

A very peculiar and at the same time interesting phenomenon caused by the growth of the mycelium of the fungus in the shoot is the formation of spherical-shaped witches'-brooms on trees mostly past the seedling stage. These (Pl. XXXII, fig. 2) brooms in old trees often assume large proportions. A single witches' broom may weigh as high

as 100 pounds and measure 5 or 6 feet in diameter. The branch supporting it will hang vertically, the broom swaying in the wind like a great bag (Pl. XXXII, fig. 3). The average size of the brooms is about 2 feet in diameter. Although a few isolated cases had been noted on the seeming association of this needle fungus with these compact brooms, it was not until the field season of 1913 that this association was found to be of common occurrence. This was all the more interesting from the fact that the cause of these formations has been a standing question with all who have seen them. In some cases they have been attributed to the yellow-pine mistletoe, *Razoumofskya campylopoda* (Engelm.) Piper, an error, however, not likely to be made by anyone familiar with the type of broom caused by this mistletoe.

The distribution of the brooms is quite general through the range of the yellow pine in the Northwest. They are particularly abundant in the vicinity of the great lakes of Idaho and in the dry valleys of southern and western Montana. Climatic variation does not seem to influence their distribution.

In order to determine the cause and nature of the formation of these brooms and the relation, if any, between them and the fungus common on their needles, the subject has been under investigation in the field and laboratory. A number of interesting observations have been recorded.

The disease caused by *H. deformans* primarily affects the needles. In young pines the disease occurs quite generally at first, unaccompanied by any kind of hypertrophy of the shoots. Later the repeated destruction of the last-formed and older needles initiates a swelling of that portion of the branch. Sometimes the entire shoot succumbs to the attack in seedlings of tender years, especially the weaker individuals, caused, no doubt, by the rapid drying out of the shoot. In growths of 7 to 10 years the fungus confined itself to the needles of the season, with the result that on the infection of these a second crop sometimes appears about the terminal bud, which may or may not become infected but may remain in a stunted, deformed condition. They help, however, to maintain the shoot in a living condition. In a far greater measure than in any other member of the order the mycelium of *H. deformans* penetrates the leaf sheath and eventually perennates in the tissues of the shoot, causing a marked enlargement of the parts infected. The fungus, however, fruits only on the needles.

An additional result of the infection of the terminal shoots and the continued production of food materials by the older, uninfected needles is the stimulation of all lateral and adventitious buds either between the primary terminal buds or at the last two or three nodes. Eventually, the food materials are more and more diverted from the main shoot, resulting in a gnarled and curved bunch of short branches. Young trees

4 to 8 years old when uniformly infected are frequently observed with the terminal portions of every principal branch in the process of "brooming." The fact that the fungus sometimes occurs without the least sign of a hypertrophy of the branch does not indicate that it is not capable of producing such physiological and morphological changes. The fact remains that on all young growth almost always the twigs bearing the infected needles are abnormally swollen or branched. The fungus has not been found by the writer on mistletoe brooms or on any form of broom caused by insect or other animal injury. On large and mature trees *H. deformans* very rarely occurs on any part of the tree except the needles of these brooms. These abnormalities are scattered promiscuously over the tree, but principally on the lower branches. This indicates the nature of an infection. The more recent infections on old trees are usually distributed or isolated on particular branches. Serious injury seldom results from the growth of the brooms on more mature growth. Very rarely may the brooms become so heavy as to split-off the supporting branch.

As the result of an examination of the witches' brooms on yellow pine in the Bitter Root and Missoula River valleys, Montana, and the Coeur d'Alene region of Idaho, with respect to the presence of *H. deformans* on the brooms and the number, position, and distribution of the brooms on the tree, the following data were obtained:

On 107 trees examined, the average number of witches' brooms per tree was 3.2. These brooms generally appeared on the lower part of the crown on the side facing the prevailing winds. The average number of brooms per tree bearing needles showing apothecia of *H. deformans* was also 3.2.

These figures support the view that the peculiar brooms so common on yellow pine are the result of fungus infection and that the fungus responsible is *H. deformans*.

In the parts of northern Washington, Montana, and Idaho so far visited, *H. deformans* has not been found to attack the yellow-pine reproduction in as great a degree as in regions farther to the west and south. This is probably due to a greater mixture of species. The fungus is not able to spread with the same rapidity as in the more typical yellow-pine stands. The infected young growth usually continues alive indefinitely, and deformed branches appear, eventually resulting in an entire retardation of growth, and finally die. This process may require several seasons, but the infected pines never attain a very large size. Such deformed trees usually are attacked by bark beetles, such attacks hastening their decline.

In parts of Oregon in the yellow-pine belt the fungus was found to be very destructive. During an investigation of the larch mistletoe (*Razoumofskya laricis* Piper) in the vicinity of Sumpter, Oreg., the

yellow-pine reproduction, especially on south slopes under mature cover, was observed to be turning brown and in many cases dying. On examination the needles of these seedlings showed that they were infected with *H. deformans*. This is a grazing region, and the forest has been continuously grazed by large bands of sheep for many years. The stems of the young pines in numerous cases bore near their bases one or more wounds of a shape and nature indicating that they were produced by the treading of grazing animals. Since little information is at hand on the effects on forest production of wounding by grazing animals, it seemed worth while to make a detailed study of the case so far as time would allow, with the double object of determining which injury—viz, the needle fungus or the wounding—was responsible for the sickly condition of the young pines. It must be remembered, however, that the seedlings were growing under the canopy of a mature yellow-pine stand; consequently they were not growing rapidly in height. Four one-tenth-acre plots were laid off on representative south slope sites and every seedling on the plots carefully pulled up and bound in bundles. These bundles were sent to the laboratory and afterwards carefully diagnosed. The normal condition of root system and crown and general vigor of seedlings were judged from a knowledge of normal young pines of the same age, free from disease and wounds, growing in the same regions and under the same slope conditions. The results of this study were embodied in a preliminary table from which Table I was condensed as being more readily undestandable.

TABLE I.—*Number of seedlings on 4 one-tenth-acre plots, average age and height, condition of infection with Hypoderma deformans, present condition of wounding and root system, south-slope type*

| Condition of seedlings. | Number of seedlings on 4 one-tenth-acre plots. | Average age. | Average height. | Vigor and general appearance of seedlings. |
|---|---|---|---|---|
| | | *Years.* | *Feet.* | |
| Seedlings neither wounded nor infected. | 40 | 10.7 | 2.8 | Healthy green color, vigorous, normal, well developed root system. |
| Seedlings infected with *Hypoderma deformans* but not wounded. | 67 | 13.0 | 1.3 | Few green needles, most all badly infected and either dead or dying; twigs twisted or broomed; poorly developed root system; general picture of a starving condition. |
| Seedlings both wounded and infected. | 127 | 12.1 | 1.4 | Do. |
| Seedlings wounded but not infected. | 49 | 12.8 | 2.14 | Wounds mostly healed. Time required to heal, 1 to 4 years. Seedlings normal. Wounding apparently not affecting growth. Root system normal. |

## DISTRIBUTION OF HYPODERMA DEFORMANS

The disease of yellow-pine needles caused by *H. deformans* is widely distributed throughout the northwestern part of the United States and western Canada. Its distribution in other parts of the West is not known, although the fungus has undoubtedly been collected

by other observers.[1] The writer has observed and collected *H. deformans* in the National Forests of the Northwest as follows: Sioux, Helena, Deerlodge, Jefferson, Missoula, Coeur d'Alene, St. Joe, Clearwater, Selway, Bitterroot, Pend Oreille, Kaniksu, Nez Perce, Lolo, Cabinet, Flathead, Kootenai, and Whitman. The late J. F. Pernot, forest examiner, supplied specimens from the Deschutes, Wallowa, Malheur, Crater, Colville, and Wenatchee National Forests. Along the Thompson River in British Columbia the fungus was occasionally found by the writer in the summer of 1913.

## CONCLUSIONS AND RECOMMENDATIONS

A very conspicuous disease on yellow-pine needles in many parts of the Northwest, the cause of which has for several seasons remained unknown, is found to be caused by a fungus which is described as a new species under the name "*Hypoderma deformans*."

*H. deformans* is a true parasite and attacks the foliage of all age classes; and in some of the more exposed sites of the typical yellow-pine belt of Montana, Oregon, Washington, and Idaho, young seedlings at first suffer great suppression and are finally killed.

The first sign of infection of the needles is usually a slight browning of the tips; or in the regions of heavy infection the entire needle may gradually assume a straw-yellow color, deepening to a brown on the first appearance of the apothecia.

Because of the destruction of the youngest needles and the penetration of the mycelium of the fungus in the tissues of the stems of the host, the terminal shoots do not attain their proper development, but become stunted and deformed, eventually producing a witches' broom. These witches' brooms on young yellow-pine saplings or older trees are often very conspicuous and often occur in such numbers as to make either an individual tree or an entire stand look very ragged and unsightly.

Up to the present time the disease has not been found in the forest nursery, but it may be regarded as a possible nursery disease. Since the vegetative mycelium of the fungus may hibernate in the shoots of seedlings after the infected needles have fallen, the fungus may make its appearance in the forest nursery and may be unknowingly transferred to the planting areas.

The presence of the fungus on mature forest trees is very readily recognized by the foliage browning up in patches or by the formation of brooms. Since the fungus does not affect the merchantability of the tree, except by influencing the increment in cases of very severe infection, all trees of the regulation diameter classes should be marked for

[1] Meinecke describes a very destructive needle fungus, under the name "Hypoderma," on yellow and Jeffrey pines, which apparently is the same fungus as the one described in these pages. (Meinecke, E. P. M. Forest Tree Diseases Common in California and Nevada, p. 34. Washington, 1914. Pub. by U. S. Dept. Agr. Forest Serv.)

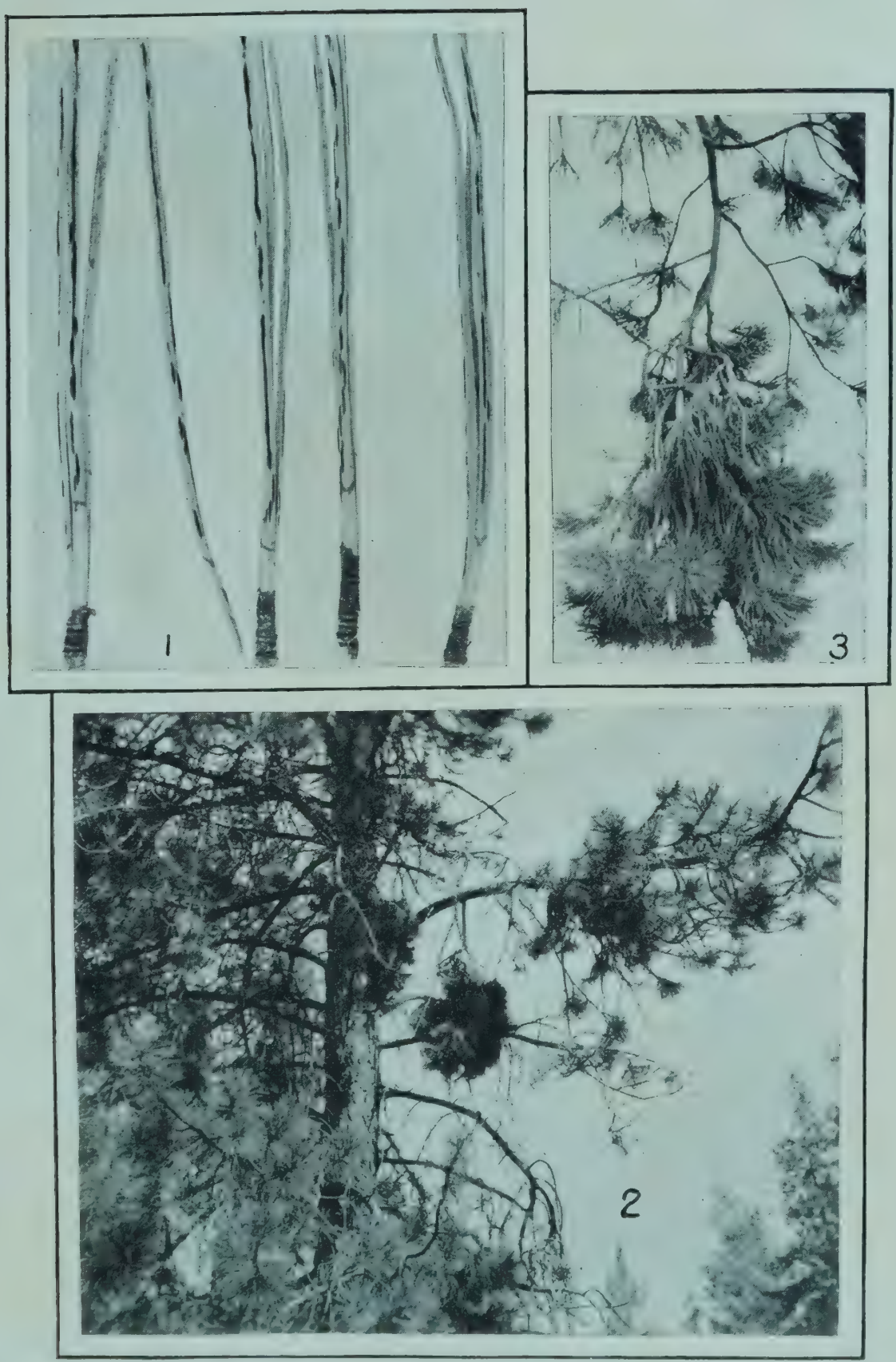
cutting. The brooms never produce cones and the normal parts of the supporting branch are usually sterile. The branches bearing patches of infected needles or brooms should be piled and burned as soon as possible. This may be done in the course of the regular brush-piling operations. If young trees below the regulation cutting diameter are so badly "broomed" that in the opinion of the forest officer the increment of the tree will be seriously impaired, and whenever the cost is not prohibitive, such trees should be lopped and immediately burned. The chief reason for such procedure is to protect the reproduction from infection, thus insuring a healthier forest in the future.

## PLATE XXXII

Fig. 1.—Needles of *Pinus ponderosa* infected with *Hypoderma deformans*, showing the apothecia. Natural size.

Fig. 2.—Branches of *Pinus ponderosa* deformed and broomed by *Hypoderma deformans*.

Fig. 3.—A branch of *Pinus ponderosa*, showing how it will hang vertically when supporting a large broom caused by *Hypoderma deformans*.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# ORNIX GEMINATELLA, THE UNSPOTTED TENTIFORM LEAF MINER OF APPLE

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## INTRODUCTION

The small, unspotted tentiform leaf miner (*Ornix geminatella* Pack.) has been extremely abundant in Missouri in recent years and has attracted the attention of fruit growers throughout the State. It has confined itself largely to bearing apple (*Malus sylvestris*) orchards, though considerable injury has been done to apple foliage in nurseries. Fortunately, it is most abundant in the late summer and early fall, so that its work is of less importance to the trees. As with many insect pests, it seems to run in cycles. It was most abundant during the summers of 1911 and 1912, reaching a climax in 1912. Since 1912 it has attracted little attention.

It confines its work to the leaves and spends most of its larval life inside the leaf as a true miner. The caterpillar therefore is small, though the characteristic elevated, or tentiform, dead patches which it produces on the leaves are quite noticeable. In some cases as many as 15 mines have been found on a single large apple leaf (Pl. XXXIII, fig. 14, 15). The pest was so abundant and so widely distributed throughout the State that a careful study of its life history, habits, and control was undertaken.

## HISTORY OF THE PEST

The moth was first described and figured by Packard (7, p. 353)[2] in 1869 as *Lithocolletes geminatella*. The description and figures are incomplete and not entirely accurate, owing perhaps to incomplete observations. Since its first discovery it has been collected by various workers and was redescribed by Chambers (2) as *L. prunivorella*. Other closely related micros have been mistaken for it, and some careful observers have given very inaccurate descriptions of its work and habits.

## DISTRIBUTION OF THE LEAF MINER

Packard reported it as being abundant in New England on pear and apple; Lowe (6) reported it as being very abundant on apple in New

[1] The writer wishes to acknowledge his indebtedness to the late Miss Mary E. Murtfeldt, of Kirkwood, Mo., to Miss Annette F. Braun, of Cincinnati, Ohio, and to Mr. August Busch, of the Smithsonian Institution, Washington, D. C., for assisting with the naming of the leaf miner; and to Dr. L. O. Howard, Chief of the Bureau of Entomology, and to Mr. A. A. Girault, of the same Bureau, for the determination of the parasites. He is also especially indebted to Prof. C. R. Crosby, of Cornell University, for helpful suggestions and for assistance in naming the leaf miner and the parasites.

[2] Reference is made by number to "Literature cited," p. 295.

York, and Brunn (1) reported it from Ithaca, N. Y. Forbes (4, p. 57) reported it from Illinois, New York, Colorado, Kentucky, Michigan, and Massachusetts; and Jarvis (5, p. 49) reported it as being common in Connecticut. Dietz (3) reported it from the Middle and Northern States of the Atlantic slope, though he confused species. In a recent attempt to determine its present distribution the writer has been able to get definite records from but one additional State, Ohio. It is probable that it is found from the Atlantic States to Colorado, but being so small and inconspicuous, except when abundant, fruit growers and entomologists have overlooked the insect and its work.

## LIFE HISTORY OF THE MINER

The writer has not been able to find any report of the complete life history of the pest. Such records as are available deal with the insect and its development and work in the summer or more often for a short period in the late fall. In some cases very careful data have been recorded, but many of the records and descriptions are decidedly at fault. The following records for the insect in Missouri have been collected since the summer of 1911 and include new data on the life history, development, and habits of the pest.

### EGG

The egg is extremely small, slightly oblong, varying from 0.254 to 0.4 mm. in length and from 0.18 to 0.29 mm. in breadth, only slightly elevated and firmly cemented invariably to the lower surface of the leaf. (Pl. XXXIII, fig. 3.) It is so small that it can scarcely be detected with a hand lens, and the writer has failed to find the unhatched eggs on foliage, though many have been collected and studied soon after hatching, when the young caterpillar had just begun to start its mine. The adults have refused to lay eggs in captivity in small vials; therefore, these records are for the freshly hatched eggs.

### THE LARVA

On hatching, the larva is footless and resembles a microscopic flat-headed borer. It always seems to break through the part of the shell which is cemented to the leaf and enters the tissue of the leaf at once. The freshly hatched caterpillar is less than a millimeter in length. It grows rapidly and when mature is about 6 mm. in length. In its development it passes through four distinct larval stages. There is considerable variation in size, but the following measurements are the average of many specimens.

In the first stage the caterpillar is pale, with a slight yellowish tinge to the head. The head and thorax are enlarged and it is footless. It molts when it is yet less than 2 mm. in length, and the head capsule is about 0.18 mm. in breadth. (Pl. XXXIII, fig. 4.)

In the second stage the body is pale, the head becomes brownish, a black blotch begins to appear on the first thoracic segment, legs are still absent, the head capsule is about 0.27 mm. broad, and the caterpillar is about 2.2 mm. long. (Pl. XXXIII, fig. 5, 6.)

In the third stage the body is at first pale, but darkens with age; the thoracic and abdominal legs appear; the thoracic blotch breaks up into four irregular spots; the head becomes darker and is about 0.35 mm. in breadth, while the caterpillar is about 4.5 mm. in length. (Pl. XXXIII, fig. 7.)

In the fourth stage the caterpillar is about 6 mm. long and the head capsule is 0.49 mm. broad; the body takes on an olive-gray color, sharply contrasting with the conspicuous white tubercles; the head becomes darker, and along its hind margin appears a row of four small black spots which parallel the similar row of larger spots on the first thoracic segment. (Pl. XXXIII, fig. 8, 9.)

### THE MINE

While the caterpillar is changing from a pale, flat, footless, microscopic caterpillar to a conspicuously marked, cylindrical, active one, its mine also undergoes distinct changes. At first the mine is serpentine in form; but after it is from 4 to 8 mm. in length and is usually curved upon itself, the caterpillar begins to transform it into a blotch mine. (Pl. XXXIII, fig. 13.) The blotch mine begins by the third day, and about that time the caterpillar changes to the second stage. At first the blotch appears only on the lower side of the leaf. The lower layer of the leaf is separated from the upper by the flat caterpillar, and soon the severed lower layer dies and turns brown. The mine remains in the blotch stage about four or five days, and during that time the caterpillar changes to the third stage. When complete, the blotch is from 1 to 2 cm. long and usually occupies all the space between two of the main lateral veins of a leaf. On preparing to produce the tentiform mine, the caterpillar spins silk threads on the floor of the mine, which causes the lower dead layer of the leaf to become folded lengthwise of the mine. These threads, with others spun later under the roof of the mine, cause the upward projection of the mine. Just about this time the caterpillar changes to the fourth stage and begins to feed on the chlorophyll cells, and this in time gives the unspotted effect when a clear net work of veins appears. During the latter part of June it was found that in from a week to 10 days after the young caterpillar begins to feed, the mine is changed from the serpentine through the blotch to the tentiform type. The majority of the feeding and growth occurs in the third and fourth stages, and after the tentiform mine is made it requires from four to seven days to eat out all the chlorophyll cells and give it the completed, unspotted, tentiform appearance. The larval life in the mine is therefore about two weeks. The caterpillar leaves

the mine through a small hole in the floor of the mine and after crawling about for a varying length of time prepares to make a cocoon in which to pupate.

### COCOON

The cocoon is almost invariably made on the upper surface along the edge of the leaf or at its very tip. On preparing to make the cocoon the caterpillar first rasps off and eats a patch of the surface layer of cells along the edge of the leaf, about 4 mm. wide and twice that in length. This causes a withering of the tissue and a slight folding over of the edge of the leaf. Then begins the work of spinning silk. First a loose layer of silk threads is spun from a line about 2 mm. from the edge of the leaf to the inner edge of the patch rasped off. Then follows a second layer of threads which are drawn very tight as they are placed. The anterior two-thirds of the body of the caterpillar enters into this work with great energy and force. The caterpillar's silk press must be a strong one. This layer only slightly draws up the edge of the leaf, but after transversed bands are used to tie the tight threads in bundles the edge of the leaf is perceptibly folded. At this time a second layer of foundation threads are spun underneath and then the work of drawing tight threads is continued along one end of the future cocoon. In half an hour the leaf edge is half drawn over and the hardest part of the work is completed. After the edge is tied down tightly the inclosed space is thoroughly lined with snow-white silk, so that a very dense semicircular cocoon 8 mm. long is formed. (Pl. XXXIII, fig. 12.)

### PUPA

The mature caterpillar pupates soon after the cocoon is completed. The pupa is about 4 mm. long, exclusive of the antennal sheaths which project fully a millimeter beyond the tip of the body (Pl. XXXIII, fig. 10, 11). The pupa darkens with age, becoming dark brown on the dorsum and yellowish brown on the venter. The leg, wing, and antennal sheaths are all distinct. The pupal period varies from a few days to a week in midsummer.

### MOTH

The newly-emerged adult on assuming its full splendor is truly a beautiful creature when viewed through a microscope. When left undisturbed it will stand perfectly still for hours, with the head elevated and the tip of the wings and abdomen lightly touching the surface on which it rests (Pl. XXXIII, fig. 2). This is its characteristic pose, and it holds it so perfectly that prolonged exposures for enlargements can safely be made. While in this pose the light flashes from every properly arranged scale as from polished metal, and one who is only familiar with the appearance of museum specimens can hardly appreciate the peacock-like splendor of this seemingly proud little creature.

Brunn's (1) description of the adult is very good. To the unaided eye the moth is slate-gray with a slight tinge of brown, being lighter in ruffled specimens. The ventral surface of the body is lighter in color. The markings on the front two pairs of legs are similar. The tarsal segments are white, tipped with black; the tibia and femur vary from dark brown to black with lighter patches; the coxæ are mottled with white and dark scales. The tarsal segments of the hind legs are brownish with white basal bands, while the tibia, femur, and coxa are much lighter, being nearly the same color as the lower surface of the abdomen. The palpi are prominent and banded with white and dark scales. The brownish proboscis is unusually long, reaching to beyond the base of the abdomen which, though it has not been observed to do so, would lead one to conclude that the moth feeds. The antennæ are brownish in color and distinctly annulate with whitish. In life they are closely pressed along the sides of the body and reach to beyond the tip of the abdomen and wings.

The surface of the forewings is beautifully mottled with light and dark scales. The light scales are arranged in eight or nine more or less distinct transverse bands. In museum specimens it is difficult to distinguish these bands. Near the tip of the forewings in fresh specimens, is a distinct black patch of scales bordered without by three alternating, narrow, white and black curving bands, giving to the tip of the wings a distinct peacock spot. On the hinder margin of the front wings the black and white scales forming the terminal peacock spot give way to long, light-colored hair. This border of delicate hair ceases near the middle of the hinder margin of the wing. The hind wings are slender and armed on the hinder margin with a broad band of delicate light-colored hair. This band becomes narrower toward the tip of the wing. The costal band is scarcely as broad as the wing (Pl. XXXIII, fig. 1).

The moth has a wing expanse of from 7 to 9 mm. and is approximately 5 mm. long when at rest with the wings folded.

## NUMBER OF BROODS

This species winters in the pupa stage in a carefully prepared cocoon protected by the folded-over edge of a leaf. In the spring the adults are abundant by the first week in May. By the middle of May the typical tentiform mines begin to appear, and the adults of the first spring brood begin to emerge by the last of May. The life cycle is completed in from four to five weeks. The broods overlap, but beginning with May a fairly well-defined brood can be made out for each month until November. The larvæ of the October brood pupate and live through the winter on fallen leaves. After the moths emerge a considerable period of time elapses before the mines begin to appear. This is undoubtedly due to the fact that the moth, with its well-developed proboscis, feeds for a time before ovipositing.

## FOOD PLANTS OF THE LEAF MINER

This leaf miner is primarily a pest of the foliage of the apple. There is where it abounds. However, the small caterpillars have been found developing in considerable numbers in the leaves of the crab-apple (*Malus* sp.), and to a less extent in the leaves of the haw (*Crataegus* spp.), plum (*Prunus* spp.), cherry (*Prunus* spp.), and pear (*Pyrus* spp.). In the case of the last four trees only an occasional mine has been observed. Chambers (2) and others have also reared it from mines in the leaves of the wild cherry (*Prunus* spp.).

## CONTROL OF THE LEAF MINER

While this miner may develop in such numbers that from 90 to 95 per cent of all leaves on apple trees may contain from 1 to 10 or 15 mines, it must be said that it is not an especially alarming pest of the orchard (Pl. XXXIII, fig. 14, 15). The pest increases in abundance as the summer and fall advance, so that by September or October much of the foliage may be consumed, but by that time the tree has about completed its growth and matured its crop. However, when conditions are favorable and the pest is abundant, steps should be taken to prevent it from reappearing in injurious numbers the next season.

Since the caterpillar enters the leaf immediately on hatching and remains in the mine until mature and ready to spin its cocoon for pupating, arsenical and contact sprays are of no value. Applications of sprays have given the writer absolutely no results. From the general nature of the pest and its habits, there seems to be no feasible means of controlling it during the growing season. Since it passes the winter as the pupa in cocoons on fallen leaves, it can be effectively controlled by destroying the leaves early in the spring. The most practical method of destroying the pupæ on the leaves is to use a disk for shallow cultivation before the first of March so as to work under the leaves before the moths begin to emerge. Summer cultivation will not help, since the pest is not found on the ground at that time. In a small home orchard the leaves can be raked together and burned or piled and used for leaf mold. If they are not burned, they should be covered with enough soil or stable manure to hasten the decay of the leaves and prevent the moths from emerging in the spring.

## PARASITES OF THE TENTIFORM LEAF MINER

It would seem that a caterpillar of this type, which lives protected inside the leaf from the time it hatches from the egg until it is ready to pupate, would be as well protected from natural enemies as from artificial treatment given by man. This does not prove to be the case, however, for the pest is heavily parasitized. It resembles other insect pests which are subject to the attacks of parasites in that under favorable

conditions it increases rapidly and then when the parasites get the upper hand it suddenly disappears. In the summer of 1912 it reached a climax as regards abundance. During the fall the parasites increased in such numbers that but few of the caterpillars escaped to pupate and pass the winter. The check, owing to the beneficial work of the parasites, was complete, for the miner has not attracted attention since 1912.

As the investigation of the miner progressed, it was observed that many of the mines went no farther than the blotch stage, while others arrived at the tentiform stage; but from them no caterpillars emerged. In such mines would be found the dried skin of the caterpillar and the larva or pupa of a parasite. Only casual observations were made on the habits and life cycles of the different species of parasites. One of the common species was found to attack the more mature caterpillars and pupate in a small, oval, white cocoon suspended in the tentiform mine. Others destroyed the younger miners and pupated without producing cocoons in the blotch mines. The grub of one of the parasites was observed to attack the miner just behind the third pair of thoracic legs, paralyzing and eventually destroying it.

The collection of parasites was first submitted to Prof. Crosby, who, from a portion of the collection, identified two species: *Sympiesis nigrifemora* Ash. and *S. tischerae* Ash. Later Mr. Girault examined the collection and identified two new species, *S. meteori* Girault and *Eulophus lineaticoxa* Girault, and one previously recognized species, *S. dolichogaster* Ash. Besides these five species, there were a number of males which were not determined. Brunn (1) reared two species of Sympiesis from the mines of this insect. They were recorded under the manuscript names of *S. minutus* Howard and *S. lithocolletidis* Howard; but the descriptions by Howard were apparently never published, and Ashmead later redescribed the latter species as *S. nigrifemora* Ash.

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## PLATE XXXIII

*Ornix geminatella* Pack.:

Fig. 1.—Moth expanded. × 10.

Fig. 2.—Moth at rest on leaf. × 2½.

Fig. 3.—Egg on lower surface of leaf; also tunnel made by miner on leaving the egg. × 80.

Fig. 4.—Dorsal view of first larval stage; below, side view of head and thorax. × 18.

Fig. 5.—Dorsal view of second larval stage. × 18.

Fig. 6.—Side view of second larval stage. × 18.

Fig. 7.—Dorsal view of third larval stage, showing edge of thoracic legs. × 18.

Fig. 8.—Dorsal view of fourth larval stage. × 18.

Fig. 9.—Side view of fourth larval stage. × 18.

Fig. 10.—Ventral view of pupa. × 18.

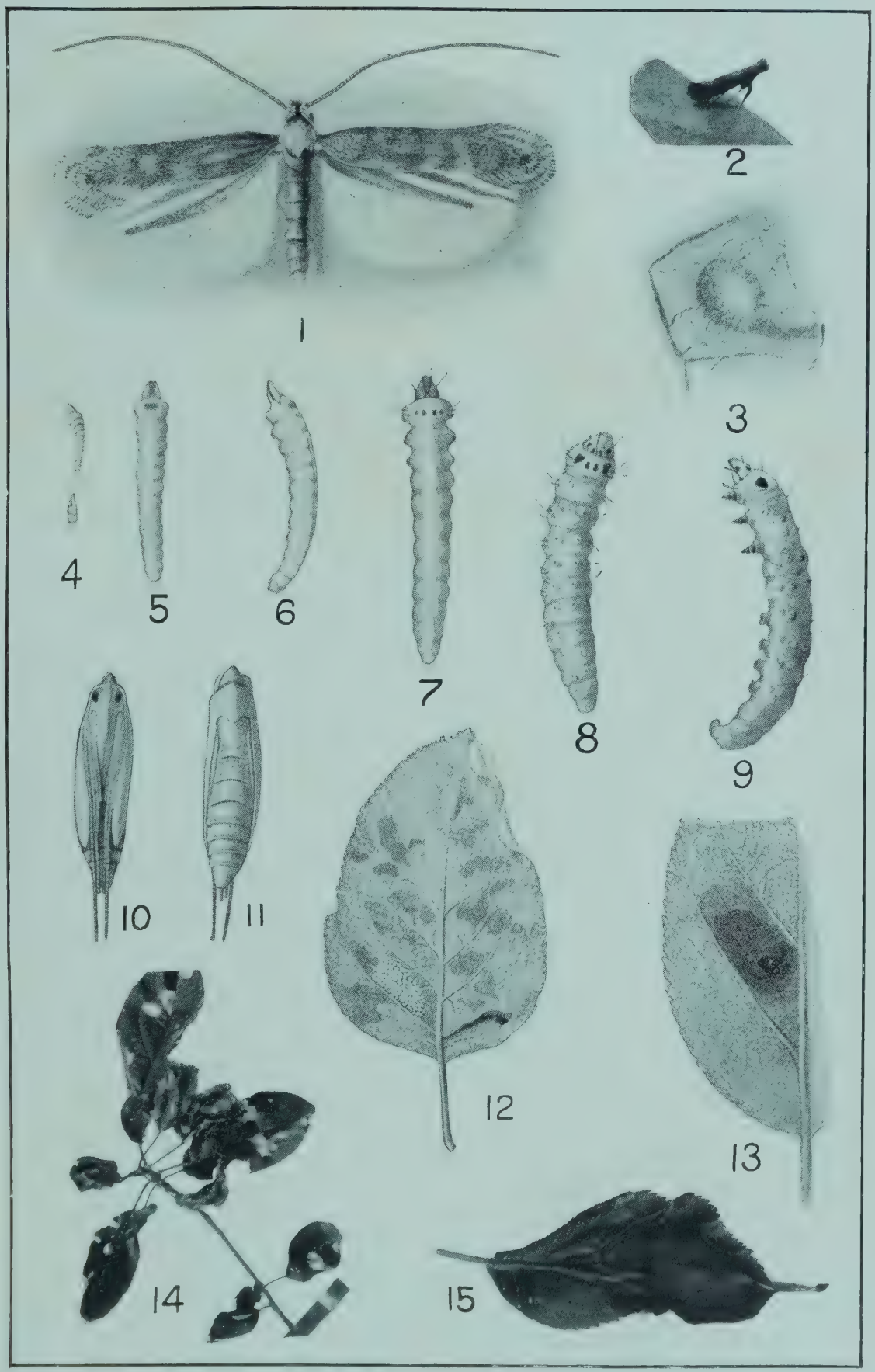
Fig. 11.—Dorsal view of same. × 18.

Fig. 12.—Lower surface of leaf with numerous partly developed mines; also two cocoons, one exposed. The cocoon is usually on the upper surface of the leaf. × 1.

Fig. 13.—Portion of leaf showing a mine in process of development. The serpentine mine was completed on June 24, the small darkly shaded area of the blotch mine June 25, the second area on June 27, the third area on June 29, and on June 30 the blotch was completed and then transformed to the tentiform mine. × 2. Egg more enlarged.

Fig. 14.—A small twig showing leaves badly curled and injured by numerous mines. × ¾.

Fig. 15.—Leaf much distorted with 10 mines almost completed; also one cocoon appears at the tip of the leaf. Natural size.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# SILVER-SCURF OF THE IRISH POTATO CAUSED BY SPONDYLOCLADIUM ATROVIRENS

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## INTRODUCTION

Silver-scurf of the Irish potato (*Solanum tuberosum*), caused by *Spondylocladium atrovirens*, has been known in Europe since 1871, when it was discovered by Harz (6) on new potatoes in Vienna; but there is no record of its appearance in this country until mentioned by Clinton (4) in 1908. Notwithstanding its comparatively recent discovery, its general distribution in the eastern United States was shown by Melhus (7), 1913, who also raised the question as to its importance as a new potato disease in America, while its appearance in the Northwest was first reported in 1914 by Bailey (2) and later, in 1915, by O'Gara (8).

Reports of studies made by former investigators contain contradictory assertions, especially on the effect of this organism upon the host. It is evident, therefore, that further study of the symptoms, manner of infection, and physiology of the organism is desirable in order to understand more fully the significance of this disease, which has already become widely distributed in this country.

## STUDIES OF THE FUNGUS

### MORPHOLOGY

*Spondylocladium atrovirens*, one of the black molds, is classified according to Saccardo (9, p. 483) in the Fungi Imperfecti under the Dematieae. The genus Spondylocladium is characterized by its dark multiseptate conidiophores, which bear the many-celled conidia pleurogenously in the form of whorls.

Conidiophore and conidia formation can be studied either in hanging-drop or agar cultures. When the organism was cultured on agar plates

[1] The sincere thanks of the author are due to Dr. I. E. Melhus, Bureau of Plant Industry, for many helpful suggestions during the progress of this study and the preparation of the manuscript, and also to Prof. L. R. Jones, of the University of Wisconsin, in whose laboratory a part of the work was conducted.

held at room temperature, conidiophores and conidia appeared in 10 to 12 days, which indicates that *S. atrovirens* is one of the slow-growing fungi.

The conidia are formed first either at the apex or the distal end of the intermediate cells. Under certain apparently abnormal conditions, however, they appear at the ends of what seem to be ordinary branches of the mycelium, but in that case the character of the normal conidiophore is absent. The lowest whorls of conidia are borne about halfway between the base and the apex of the conidiophores, and the conidia are attached at the broad end (Pl. XLVI, fig. 2).

Germination of the conidia takes place by means of germ tubes. These are produced from either pole, generally from the distal or pointed end, as well as from any cell of the conidium, as observed by previous investigators. Germination in water occurs within 24 to 40 hours; and in a few days the somewhat hyalin, knoblike protrusion, which is characteristic of the early stages of germ formation, develops a multiseptate, branched mycelium which is of a much lighter color than the conidiophores, conidia, or portions of the old mycelium. This is very hyalin and continues so up to the time of conidiophore formation, at which time dark-brown, thickened cells are formed in different parts, and from these specialized cells are produced the many-septate, dark-brown conidiophores, which attain a length of 5 mm. and are perceptibly wider than the surrounding mycelium (Pl. XLVI, fig. 1).

Because of the wide variation found in the size of the spores, Appel and Clinton (4, p. 359) suggested the possibility of there being two species of the fungus—that is, a large-spore and a small-spore species. Several series of 18 measurements were made by the writer on conidia taken from tubers imported from Germany and tubers from various parts of the United States. A wide variation in dimensions occurred in the conidia from all the various tubers used in the experiment. The conidia taken direct from the surface of the tuber from Germany varied from 22 to 42$\mu$ (mostly 30 to 40) in length, 6 to 12$\mu$ (mostly 6 to 8) in width at greatest diameter, and were 4 to 8 (mostly 5 to 6) septate; conidia taken from the progeny of tubers from Maine grown in Washington, D. C., varied from 30.4 to 56.2$\mu$ (mostly 30 to 40) in length, 7.6 to 9.5$\mu$ (mostly 7.6 to 8.5) in width, and were from 4 to 7 septate; while the conidia taken from tubers from Rhode Island, West Virginia, Washington, D. C., Oregon, Washington, and Wisconsin averaged 32.6 to 40$\mu$ in length, 7.5 to 8.5$\mu$ in width, and were 5- to 7-septate.

In order to study more fully the variation of spore dimensions, several series of measurements were made on conidia produced from a single spore strain. The difference in dimensions obtained in this case ranged from 18 to 64$\mu$ (mostly 30.4 to 40) in length, and 7 to 8.1$\mu$ in width, and 5 to 6 septa.

From this it is apparent that, even though considerable variation in spore dimensions occurred on infected tubers from different localities, nevertheless an even greater variation resulted in the case of spores from a single spore strain. This shows that normally a wide variation exists, and consequently it does not appear necessary to form small-spore and large-spore species.

## REACTION OF THE FUNGUS TO LIGHT

In order to secure a better knowledge of the relation of *S. atrovirens* to its environment so that its life history might be better understood, experiments on some of the physiological characteristics of this organism were conducted.

The reaction to light is of special interest in connection with the effect of storage conditions upon the development of the fungus on potatoes.

In this study the writer used the plate-dilution method, the conidia being sufficiently diluted on Lima-bean agar plates to be observed individually. Immediately after the plates were poured, each was wrapped in carbon paper, the entire dish being covered except an aperture from 1 to 2 cm. in diameter at the side, and the plates were then arranged with the apertures facing the light from the window.

The plates were examined at the end of three days and it was found that the mycelial branches developed on the side of the hyphæ farthest away from the window and that the majority of these grew in the opposite direction from the source of the light. The position of germ-tube formation does not appear to be influenced by the light, germination sometimes taking place from the side closest to the source of light; but as soon as the germ tube receives the heliotropic stimulus—that is, when it is a few millimeters long—it invariably turns away from the light, and subsequent mycelial development is formed on the side of the conidium farthest from the source of light. Instead of appearing at the center of the colony, therefore, the conidia are found at the margin exposed to the light, and at the end of 5 to 10 days the entire colony appears as if a gentle breeze had blown the hyphæ in one general direction away from the light (Pl. XLVI, fig. 3). These results also confirm Eichinger's (5) observations.

The reaction of this fungus to light in culture media demonstrated that it is negatively heliotropic. In view of the fact that infection of the tubers in the field takes place in the dark, negative heliotropism here does not obtain. In order to determine whether this heliotropic property favored tuber infection, artificial inoculations were made on tubers in the light. In this case no perceptible difference occurred, since infection appeared on all parts of the tubers alike.

## REACTION OF THE FUNGUS TO MOISTURE

Like most fungi, *S. atrovirens* requires considerable moisture for development; but, owing to the absence of accurate instruments for

measuring the degree of moisture, only approximate data regarding moisture reaction can be given. It was noted in field studies that a higher percentage of infection occurred in the lower and more moist sections of the field than in the higher areas, and that in laboratory infection experiments the fungus develops best when the surface of the tuber is kept moist but not supersaturated. By placing tubers sufficiently near water so that a heavy film of moisture was constantly present, it was found that sporulation was inhibited to a greater degree on the side of the tuber near the water than on the opposite side, which indicates that excess moisture may check the growth of the fungus.

Although the fungus prefers moisture for growth, it can withstand drying without the entire loss of its virility. This was shown by the fact that transfers from agar cultures 16 months old continued to grow, although only a small percentage of the conidia germinated. Notwithstanding the fact that these cultures had been kept at room temperature and were dried to such an extent that simply a dry, brittle mass of media and fungus remained, both viable conidia and mycelium were found.

### REACTION OF THE FUNGUS TO TEMPERATURE

Conidia in corn meal and oat agar and in water and naturally infected and artificially inoculated potato tubers were used in studies to determine the effect of temperature on *S. atrovirens*. In the case of media spore-dilution plates were prepared, the spores being sufficiently far apart so that individual colonies were retained. The same dilution was used on each plate and all were inoculated at temperatures ranging from 2° to 31° C. The water cultures were used in making hanging-drop preparations on Van Tiegham cells and in small Petri dishes, the spore suspensions in this case also being made in such manner that some of the spores remained on the surface, although germination occurred to a slight extent also beneath the surface. The naturally infected and artificially inoculated tubers were placed in pint bottles containing some pebbles and a few cubic centimeters of water, with a piece of cheesecloth extending from the contents of the bottle to its mouth, thus forming a moist chamber. These bottles were incubated in the same way as the media cultures.

In the eight series of Petri-dish cultures microscopic germination was noted at 3°, 4°, and 5° C., but no macroscopic colonies developed; at temperatures ranging from 6° to 28° macroscopic colonies were obtained, 21° to 27° being the optimum for abundance of growth; while at 30° or 31° no macroscopic growth was apparent (Pl. XLVII). These temperature limits for growth were confirmed by the water cultures, which were used as checks on the media cultures subjected to the highest and the lowest temperatures. In the case of three series of these water cultures which were subjected to a temperature of from −5° to −10° C. for four days and then brought to room temperature, 80 per cent of the

conidia germinated within 48 hours, and pieces of the mycelium in the cultures also showed growth. Agar culture and cultures on sweet-clover stems subjected to the same temperature also remained viable, as indicated by subsequent transfers, hanging-drop cultures showing that both conidia and mycelium retained their vitality.

In the test with naturally infected and artificially inoculated potatoes sporulation occurred on the former at temperatures ranging from 6° to 27° and on the latter at a range of from 12° to 27° C. In cultures on agar media and sweet-clover stems subjected to 35° and 50° further growth was inhibited at the former temperature, but the fungus remained alive after two weeks' exposure, while it was killed when subjected to 50° for three days.

### REACTION OF THE FUNGUS TO MEDIA

Since *S. atrovirens* is a relatively slow-growing organism, tests were made with media of different grades of acidity with a view of facilitating growth in culture. The media used for this purpose were synthetic, Lima-bean, string-bean, oat, potato, corn-meal, and beef agar, all of which varied in reaction from +15 to −15 Fuller's scale.

Two plates each of these media equally diluted with conidia from the same culture were poured, and all were incubated at room temperature. Examinations of the colony development, including nature and extent of growth and sporulation, were made at 4-, 6-, and 12-day intervals and showed that *S. atrovirens* developed slightly faster on potato and Lima-bean agar than on string-bean, corn-meal, or oat agar; that growth was much retarded on beef agar; that mycelial development was very de cidedly inhibited on synthetic agar; that sporulation occurred slightly sooner on oat agar than on the other agars; and that the hyphæ on fruiting remained lighter in color on Lima-bean and beef agars than on other agars.

The optimum reaction appeared to depend largely on the kind of medium. On potato agar no perceptible difference in growth appeared between +10 and −10, but mycelial development was much retarded at +15. On corn-meal agar only +1, 0, −1, −3, −5, and −15 reactions were run, because of the fact that hydrolysis took place when there was a higher degree of acidity. In this series +1 reaction was the optimum for growth, and in this case the mycelium became dark earlier than was the case in the minus reactions, owing possibly to the hydrolytic action of the acid on the media. On Lima-bean agar with +5 to −3 reactions the apparent growth of the fungus was not much changed, but with 5 to 10 and −3 to −10 reactions mycelial growth was perceptibly retarded. On beef agar optimum reactions ranged from 0 to +1, very little difference appeared in the colonies at +3 to −3, growth was gradually retarded at 5 to 15, and no colonies were macroscopically visible at the end of 10 days on reactions ranging from −5 to −15.

Besides this test of different reactions of the medium, a series of nutrition tests was conducted, a full nutrient agar, including carbon, nitrogen, oxygen, hydrogen, potassium, phosphorus, magnesium, sulphur, and iron, being used. With one exception each set of the media contained one element less than the full nutrient culture; in other words, the experiment was arranged as follows: (1) Check containing water agar, (2) full nutrient, (3) full nutrient minus nitrogen, (4) full nutrient minus potassium, (5) full nutrient minus phosphorus, (6) full nutrient minus magnesium, (7) full nutrient minus sulphur, (8) full nutrient minus iron, (9) full nutrient minus carbon, (10) full nutrient minus all minerals. Two plates of each kind of agar were inoculated with conidia and two with mycelium from the same culture of *S. atrovirens*, and all were incubated in the laboratory at room temperature.

Examinations at the end of 15 and 20 days indicated that sporulation occurred only on the plates from which sugar was omitted—that is, Nos. 1 and 9—the colonies on these plates being of a light color and spreading character and from 1.5 to 2.5 cm. in diameter and that no sporulation occurred on the plates from which sugar had been omitted, the mycelium in these being dark and densely compacted and only 0.75 to 1.25 cm. in diameter.

This preliminary study of the reactions of media on *S. atrovirens* indicates that neutral or slightly acid reactions are more favorable for the growth of this fungus; that the kind of medium determines the effect of higher reactions on this organism as shown by the alkaline reactions of beef agar compared with the same reactions of potato or the other agars; that compounds in one kind of medium may be formed which are seemingly toxic, whereas in a different kind of medium the same adjustment produces no such inhibitory effects; and that the presence of 5 per cent of cane sugar in a nutrient agar inhibited sporulation, but induced dark, heavy, compact mycelial growth, while the absence of sugar caused sporulation and a more spreading mycelial development.

## HISTOLOGY

Studies were made to determine the relation of *S. atrovirens* to the potato. Both normal and affected material from the eye end of Irish Cobbler, Green Mountain, and Minnesota Triumph tubers badly infected normally and artificially was taken from the center and from the margin, that from the latter with and without lenticels or eyes. This material was embedded, sectioned, and stained according to ordinary cytological methods. From these studies it was evident that the mycelium may enter the tuber through the lenticels or between the lenticels through the epidermis.

After the fungus gains entrance the hyphæ invariably form within the cells, where they appear as a single branch of the mycelium; or they

may shorten and thicken to form a short and many-celled mass of hyphæ, from which the conidiophores subsequently arise. In severe cases of infection the cells appear to be disintegrated by the invasion to such an extent that only two or three instead of six or more cork layers remain above the living parenchyma. In experiments with potato roots grown under sterile conditions and inoculated with conidia and mycelium of the fungus, the mycelium grew on the surface, but did not penetrate the parenchyma, which indicates that the roots are less subject to infection than the tubers.

So far as the author has been able to determine, the fungus hyphæ confine their activity to the corky layers. In no case has it been found in the living parenchyma. This superficial infection causes a loosening of the corky and epidermal cell layers, so that these subsequently slough off. In this manner transpiration may proceed with greater facility and thus affect the parenchyma layers.

That *S. atrovirens* prefers this relatively heavy corky layer is further apparent from the fact that it grows very sparingly on the cut surface of the tubers where the loosened surface cells are invaded. Furthermore, its very limited presence on roots, stems, and stolons also indicates that it prefers the heavier, corky layers of the potato tuber.

## EFFECTS OF THE FUNGUS ON THE HOST

The progress of the disease after tuber infection may be divided into two stages, the early and the late. In the former the infected areas are light-brown and have a glazed appearance, the latter characteristic becoming especially pronounced when the infected surface is moistened. Sometimes the margins of these areas are slightly fimbricated. The discoloration, which is found on newly infected tubers at harvest time, is often so inconspicuous as to pass unnoticed, even on close examination, unless the tubers are washed. When infected tubers are placed in moist chambers, the brownish areas become olive-colored, owing to the formation of conidiophores and conidia. The late stage is characterized by the shrinking and shriveling of the diseased areas and sloughing off of the epidermis and may be subdivided into two stages: The spot or patch infection (Pl. XLV, fig. 2) and general infection (Pl. XLV, fig. 1). In the former slightly sunken isolated areas on the surface show the shriveling, and late in the storage season these areas become shriveled and sunken.

In the case of general infection the entire surface is covered with infected areas and the epidermal and corky layers may shrink to such an extent that distinct folds or ridges appear. In the red-skinned varieties the color is completely destroyed. This again largely only mars the appearance and not their food value, but still they must be sold at a sacrifice. Potatoes stored under moisture and temperature

conditions favorable to sporulation often become so badly infected that they become a dull-black, the tubers having the appearance of having been dusted with soot. Several such bins were observed in Maine in May and June, 1914.

In case of slight infection in the field the infected areas are often found in isolated spots close to the stem end of the tuber. This was the case in practically every infected tuber harvested from the silver-scurf experimental plot at Caribou, Me., in the fall of 1914 and coincides with the observations of Appel and Laubert (1). While no reasons for this phenomenon are given by these investigators, from experiments and observations so far made it appears that infection is brought about through contact of the stem end of the young tuber with the infected mother tuber (Pl. XLVIII). This is indicated by the fact that in many cases where there was but slight contact only small areas about the point of the stolon attachment showed infection, while in the case of extensive contact infection was more widespread. It is further indicated by the fact that only one or two tubers closest to the mother tuber showed infection in counts made when the crop was about three-fourths grown, while in counts made later, after the conidia had become generally distributed, a large percentage of the tubers were infected.

Although infection appears to take place through the stem end, both stem ends and eye ends are subject to infection, general infection of both resulting from artificial inoculations.

In view of the fact that investigators like Bohutinsky (3) have attributed to *S. atrovirens* foliage symptoms such as leafroll, mosaic, etc., inoculations upon stems, stolons, and roots of the potato plant were made, both under field and greenhouse conditions. Two distinct procedures were followed: In one set of experiments viable spores were sprayed upon the stems, stolons, and roots; in the other virile mycelium was inserted into the inoculated portions. Checks were also run. Experiments in this order were run during 1914 and 1915, and in every case the inoculated plants behaved like the checks—viz, no perceptible infection occurred—showing again the inability of this organism to invade the vine tissues of the host.

## METHODS OF DISSEMINATION

The fungus lives over by means of the mycelium, conidia, and sclerotia within the infected areas, so that under favorable conditions of moisture and temperature sporulation occurs and infection may spread even in storage. Not only do the infected tubers carry the disease to new sections, but they may carry it over from one season to another in the soil and in this way infect the new crop. This was the case in the author's field studies in Maine, viable conidia being found on the surface of

mother tubers taken on August 2, 1914, the date of the last examination, from an oat field at Houlton, in which they undoubtedly over-wintered in the soil. Many of these volunteer plants occurred in fields in which rotation had not been practiced, the deep snows which covered the ground the previous winter having protected the tubers.

Whether the fungus may live over in the soil from which the tuber host has been removed is not yet known, but that it may do so is not improbable, in view of what occurs in the case of fungi having a similar life history. Investigations to determine this point are now in progress.

Several series of experiments were undertaken to ascertain how readily *S. atrovirens* spreads from infected to healthy tubers and whether infection in this way might occur during the entire storage season. Inverted bell jars were used in these experiments to secure moist chambers which would hold a sufficient number of tubers for a satisfactory test and at the same time retain uniform moisture conditions. A wire rack of ¼-inch mesh was placed in each jar to support the potatoes and to prevent contact with the water in the jars, the inside of each jar was lined with blotting paper to conserve the moisture and prevent the entrance of excessive light, and the mouth was covered with window glass. Four varieties of potatoes were used: Rural New Yorker, Green Mountain, Irish Cobbler, and Bliss Triumph. A spore suspension of conidia which had been grown in pure culture on sweet-clover stems for four weeks was sprayed on the tubers with an atomizer, and for several days thereafter water was sprayed into the jars with the atomizer to keep the air saturated. A similar lot of healthy tubers was arranged as a check.

The first series was begun at Houlton, Me., on March 26, 1914; and within three weeks the entire surface of the inoculated tubers was covered with dark-brown conidiophores and conidia, while the checks were free from infection. Additional tests were made at Caribou, Me., on July 20, 1914; Washington, D. C., in December, 1914; Madison, Wis., on March 25, 1915; and Presque Isle, Me., on August 2, 1915. In each case infection occurred within three weeks after inoculation.

Similar infection experiments were conducted upon young tubers just harvested, as well as upon tubers still attached to the vines. In case of the tubers attached to the vines the soil was removed and a spore suspension was applied with an atomizer, whereupon the tubers were again covered with earth. Checks also were made. In each of these tests infection appeared upon tubers varying in diameter from 1 and 2 cm. to full-grown tubers (Pl. XLVII). Checks showed no infection.

From these results it is apparent that infection from *S. atrovirens* may take place at any stage in the development of the tubers and at any time throughout the storage season.

## METHODS OF CONTROL

Melhus (7) found in laboratory experiments that neither double strength of mercuric chlorid (1:500) nor formalin applied for longer than the ordinary periods would completely inhibit the development of *S. atrovirens* on the potato and that both injured the tubers to such an extent that germination was decidedly inhibited. He also found that in many cases sporulation was inhibited on the surface of infected tubers treated with solutions of mercuric chlorid heated by a method devised by him for heating the solution for brief periods at temperatures near the thermal death point of protoplasm.

In view of these results, field tests were conducted during 1914 and 1915, both in Maine and at Norfolk, Va. Infected tubers were treated in double strength and heated solutions of mercuric chlorid. In Maine the treated tubers were planted on virgin soil.

As noted in Table I, the temperature fluctuated slightly, owing to the lower temperature of the tubers than that of the solution in which they were immersed. This table indicates that there was a decrease in the percentage of infected progeny in the treated rows as compared with the check. However, in no case was there a complete control of the infection. Similar tests in 1915 also indicated that even though silver-scurf may be inhibited to some extent; nevertheless, no treatment served as a complete control.

TABLE I.—*Effect of warm solution of mercuric chlorid on silver-scurf of the Irish potato*

| Row No. | Strength of solution. | Temperature of solution. | Time of immersion. | Number of hills. | Number of hills infected. | Per centage of hills infected. | Average percentage infected hills in rows 14, 15, and 16. | Weight of healthy tubers. | Weight of infected tubers. | Percentage of infected tubers. | Average percentage of infected tubers in rows 14, 15, and 16. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Per ct.* | *°C.* | *Min.* | | | | | *Pounds.* | *Pounds.* | | |
| 13... | ..... | Control. | ..... | 78 | 40 | 51.28 | ..... | 85 | 11.25 | 11.67 | ..... |
| 14... | 0.2 | 47–52 | 5 | 63 | 29 | 46.03 | ..... | 84 | 10 | 10.13 | ..... |
| 15... | .2 | 47–48 | 5 | 93 | 6 | 6.45 | 21.04 | 102 | 1 | .98 | 4.91 |
| 16... | .2 | 45–49 | 10 | 75 | 8 | 10.66 | ..... | 75 | 2.5 | 3.21 | ..... |

In October, 1914, four pecks of tubers infected with S. *atrovirens* were subjected to a 1 to 1,000 solution of mercuric chlorid ranging from 45° to 53° C. for four minutes with a view of ascertaining the effect of treating infected tubers with that solution before storing. After treatment the tubers were placed in new muslin peck sacks and part of the lot stored at Caribou and part at Washington. At the same time separate lots of untreated infected and clean tubers were stored. On exami-

nation of these lots in June, 1915, the fungus was found fruiting on both treated and untreated infected tubers, but no infection was found on the untreated clean tubers.

As the treatments described do not absolutely control silver-scurf and as clean tubers only escaped infection, it is evident that disease-free seed should be selected in the fall and should be kept from contact with infected tubers in storage. Moreover, in view of the inhibitory effect of very low temperatures on the development of the fungus, the tubers should be stored at the lowest temperature permissible.

## SUMMARY

A study of silver-scurf of the Irish potato, caused by *Spondylocladium atrovirens* Harz, shows that, notwithstanding the wide range in spore dimensions, which led certain investigators to believe there might be a large-spore and a small-spore species in this country, there is but one species, as proved by the fact that conidia ranging from 18 to 64$\mu$ were produced by a single spore culture.

*S. atrovirens* is negatively heliotropic. This, however, does not materially influence tuber infection in nature.

Severe drying of the conidia and mycelium in agar culture at room temperature does not kill the fungus.

*S. atrovirens* withstands a wide range of temperature. Its growth is inhibited at 2° to 3° C., but it is not killed at − 10°. Its optimum temperature is 21° to 27°, maximum 30° C.

Optimum reaction to media varies with the kind used, neutral to slightly acid reactions being most favorable to the development of the fungus. Five per cent of cane sugar in nutrient agar inhibited sporulation.

The fungus enters the tuber through the lenticels or the epidermal layers between the lenticels. The mycelium invades and disorganizes the epidermal and corky layers, leaving in bad cases only one or two instead of six or more layers, thus apparently accelerating transpiration.

The disease may be carried from place to place by infected tubers, in which it lives over from one season to another, or to the succeeding crop by the infected tubers which remain in the field over the winter.

Under favorable moisture and temperature conditions potatoes may become infected throughout the entire storage season. Both old and young tubers are subject to infection.

Inoculations on living stems, stolons, and roots in the field and laboratory experiments produced no infection.

Warm solutions of mercuric chlorid have a more toxic effect on *S. atrovirens* than cold solutions.

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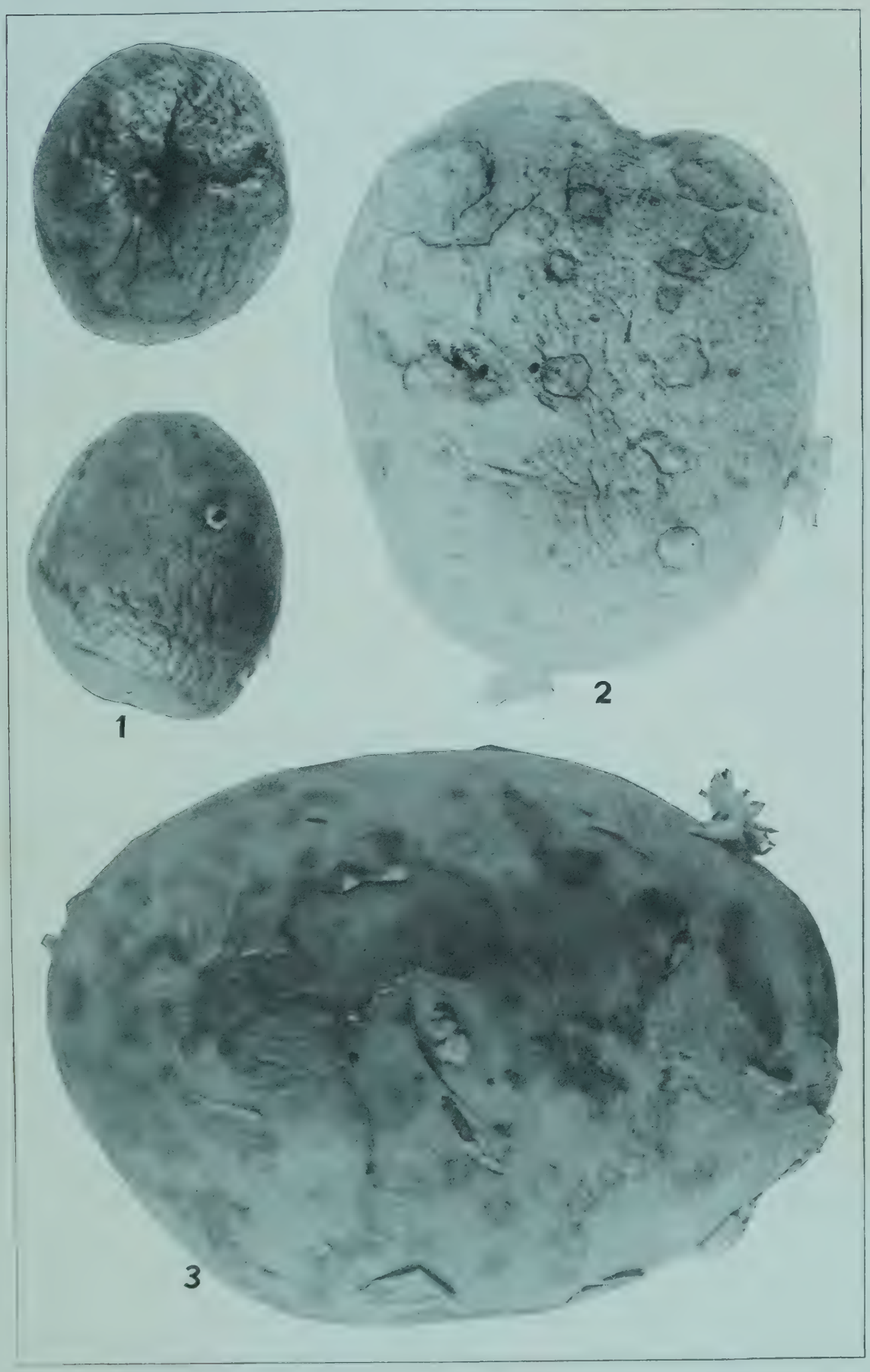
## PLATE XLV

Fig. 1.—Potato tubers showing shriveling and a silvery appearance caused by *Spondylocladium atrovirens*.

Fig. 2.—Tuber naturally infected by *S. atrovirens*, showing the segregated area type of infection, a condition developing in some cases later in the storage season.

Fig. 3.—Immature potato tuber artificially inoculated with conidia of *S. atrovirens*, July, 1913, at Houlton, Me. Infected area covered with dark-brown tufts of conidiophores and conidia. Infection was effected in a moist chamber at room temperature.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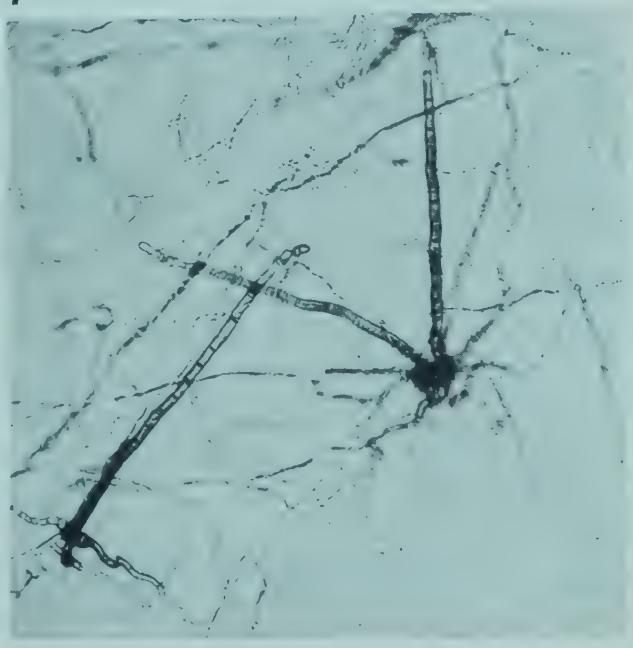

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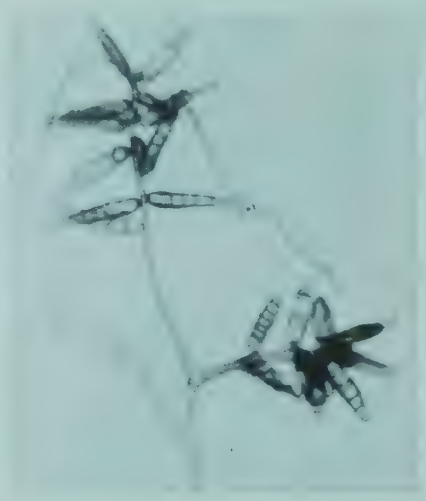

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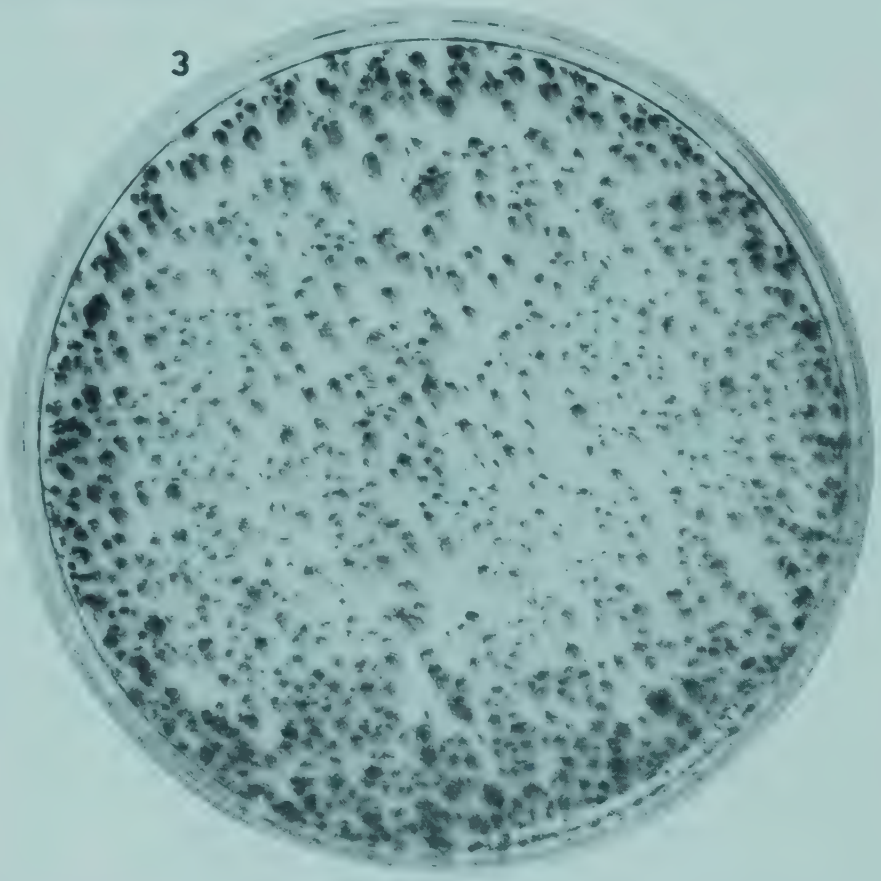


## PLATE XLVI

Fig. 1.—Photomicrograph of *Spondylocladium atrovirens* on corn-meal agar, showing method of development of conidiophores and conidia in the early stages.

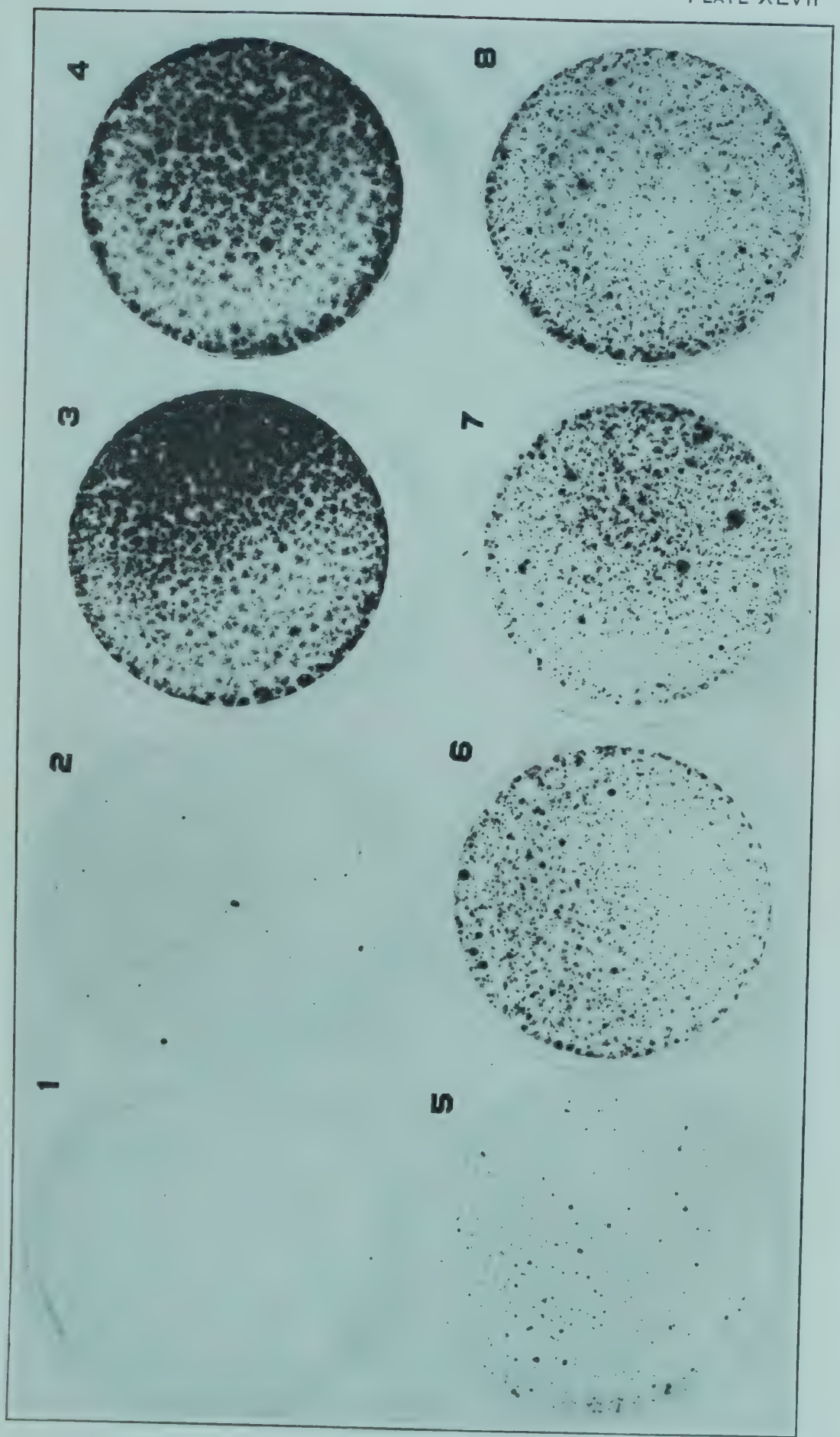
Fig. 2.—Photomicrograph of *S. atrovirens* in hanging-drop culture, showing development of conidiophore and conidia in mature stages.

Fig. 3.—Negative heliotropism of *S. atrovirens* on corn-meal agar exposed on one side to daylight from April 8 to April 24, 1915, in laboratory at room temperature.

## PLATE XLVII

Effect of temperature upon mycelial development of *Spondylocladium atrovirens* in pure culture on corn-meal agar at end of four weeks.

| Petri Dish No. | Temperature (°C.) | Petri Dish No. | Temperature (°C.) |
|---|---|---|---|
| 1 | 3 | 5 | 10 |
| 2 | 5 | 6 | 15 |
| 3 | 24 | 7 | 16 |
| 4 | 27 | 8 | 18 |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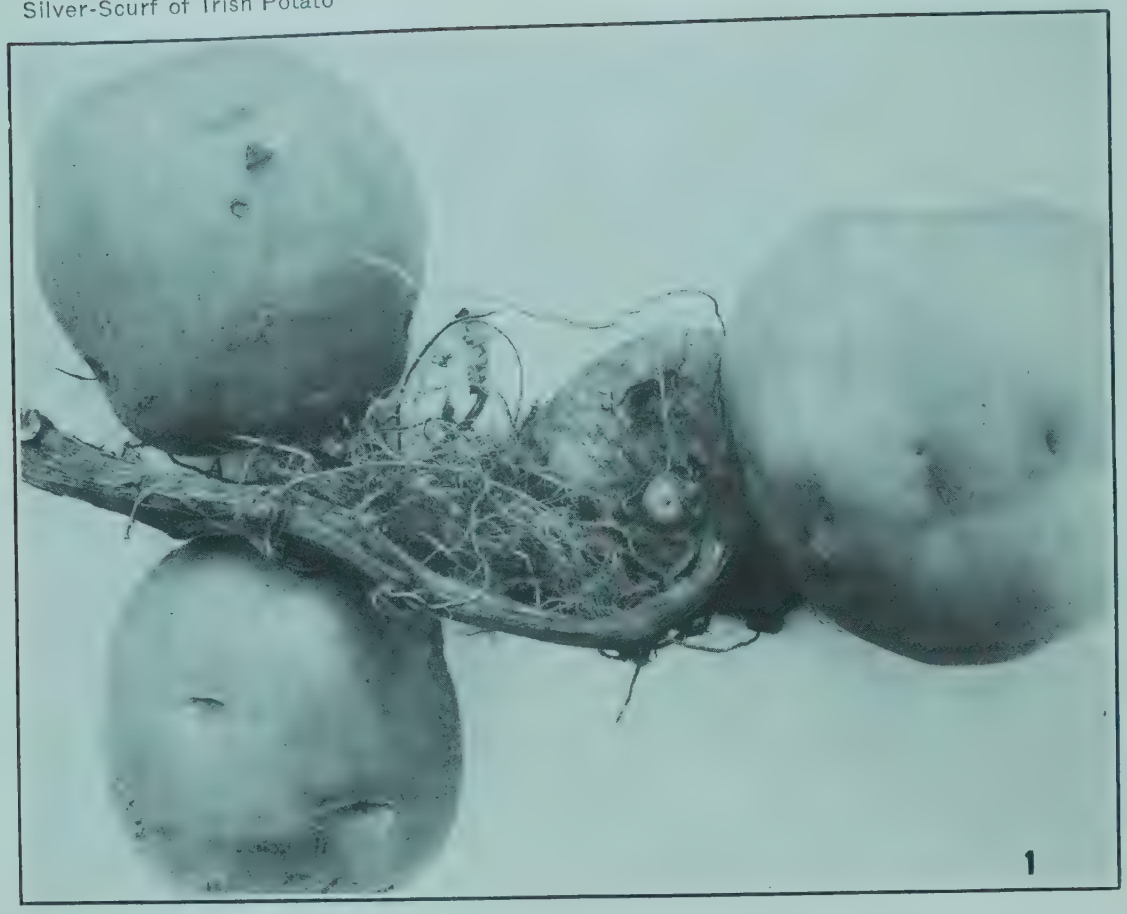
Silver-Scurf of Irish Potato



1

2

## PLATE XLVIII

Contact infection. A part of the new tubers becoming infected with *Spondylocladium atrovirens* by means of contact with the infected mother tuber. In this case it is a distinctly stem-end infection. Harvested on September 19, 1915, at Presque Isle, Me.

37767°—16——2

# WOOLLY PEAR APHIS[1]

By A. C. BAKER, *Entomological Assistant*, and W. M. DAVIDSON, *Scientific Assistant*, *Deciduous Fruit Insect Investigations, Bureau of Entomology*

## INTRODUCTION

For some years a species of Eriosoma has been known to attack pear roots in California. It has, however, been considered to be the woolly apple aphis, *Eriosoma lanigerum* Hausmann, since both in habit and in structure the two species somewhat resemble each other. To the species on the pear, which, after careful study, proves to be undescribed, the name *"Eriosoma pyricola"* is herein given, and a brief account of the species is attempted.

## HISTORY OF THE INSECT

Mr. Frank T. Swett is authority for the statement that the woolly pear aphis has been in California for more than 20 years. Ten years ago he says the species ruined about 2,000 French seedlings in one block, while occasional apple seedlings, planted along with them, made normal growth. Attention has frequently been called to the immunity of apple seedlings planted close to infested pear seedlings in nurseries and orchards.

During September and October, 1897, Mr. Theodore Pergande received specimens of a species of Eriosoma on pear roots from Prof. F. M. Webster, of Wooster, Ohio. Through the kindness of Mr. Pergande we have been able to examine these specimens, and they prove to be identical with our California material. It is quite possible, therefore, that the species may be present in other parts of the country, notably in Oregon. It is noteworthy that the Ohio specimens were taken from roots of pear stock received from France the preceding spring.

The species occurs over practically all the pear sections of northern and central California, and in some regions is a very destructive pest. To entomologists the extent of its presence has been known only for the last three or four years, but reports from orchardists and field observers indicate that it has been parasitic upon pear roots for a much longer period.

## HABITS OF THE INSECT

The insect works entirely underground. The species that has been found feeding on the aerial portions of Nelis, Easter Beurré, and other pears is the woolly apple aphis, *E. lanigerum*. The woolly pear aphis

[1] What is probably the same species has been treated as a pear pest in California under the name *Eriosoma lanigera* by Geo. P. Weldon. (The woolly aphis as a pear pest. *In* Mo. Bul. State Com. Hort. [Cal.], v. 4, no. 9, p. 441–444, fig. 94–95. 1915.)

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appears to attack the roots of all types of pears, and it is especially injurious to the French wild stock so largely used in California as a stock for the Bartlett. Quince roots are fed upon, but much less freely, and the quince may be credited with a considerable degree of immunity. The Kieffer stock is attacked, but it is possible that Japanese stock may show immunity to a satisfactory degree. Observations to date indicate that both these stocks are more resistant than that from France. It should be said that the individual plants of the wild stock from France vary greatly, and there appears to be among the plants some variation in intrinsic vigor or in power to resist the woolly aphis. However, the majority of the imported seedlings show no satisfactory evidence of a power of resistance, and a different stock is very desirable.

The insect works especially upon the smaller fibrous rootlets and may be encountered on any such rootlets within the topmost 3 feet of soil and perhaps deeper. Infestations are usually heavier on the rootlets near the trunk, but frequently the aphides are as abundant 10 or 12 feet from the stem. In a badly infested orchard the soil on being overturned may in places be found to be white with the wool and skins of the insects. The aphides attack less frequently larger roots up to ¼ inch in diameter and sometimes settle on still larger roots or on the main stem where abrasions have set up a callus growth. They often colonize the underground portions of sucker growth, feeding on the succulent stalks. After the insects have forsaken a rootlet, fungi sometimes appear and complete its destruction.

This method of feeding upon the fibrous rootlets is somewhat analogous to the habits of the grape phylloxera (*Phylloxera vitifoliae* Fitch) on the resistant types of grapevines in that chiefly the smaller rootlets are attacked. It is directly opposed to the habits of the woolly apple aphis and of the grape phylloxera on nonresistant types of vines, for both these insects feed upon the larger roots and cause the formation of tuberlike lesions. The woolly pear aphis rarely forms any perceptible lesions, but it destroys great numbers of young rootlets, especially in late summer and autumn. In old trees this sometimes results in a dwarfing of growth and in a generally unthrifty appearance and condition. The majority of old infested trees do not show evident injury ascribable to the aphis, although it is presumable that they are suffering to some extent. They remain thrifty on account of their intrinsic vigor. In many instances where old trees were showing injury, extra cultivation of the soil and better irrigation practice resulted in the establishment of thrifty conditions, even though this method did not appear to reduce the numbers of the aphis. The effect on the crop is hard to estimate and can not be satisfactorily specified, but in general it is such as may result from the diversion of the flow of sap in the tree.

With trees under 4 years of age, conditions of injury are different. Heavy infestation of a tree of weak vigor or resistance may result in the death of the tree. Badly stunted growth and the early falling of foliage are characteristic of aphis injury on young trees. Injury and death are due to heavy summer and autumn infestations on the fibrous rootlets and to the inability of the tree to replace the destroyed roots quickly enough to afford plant food for the vegetative portion. Frequently the trees are saved and relief comes from the production in the fall months of a high percentage of migrants which leave behind them for the winter only a small infestation of wingless individuals; and since the aphides increase but slowly in spring, the tree is enabled to send forth new rootlets without danger of having them rapidly destroyed. Sometimes young trees in no wise stunted have been observed to cast their leaves prematurely, and upon examination have been found to be heavily infested with the aphis. It would appear from the absence of stunted growth that these trees did not have, or were not adversely influenced by, an infestation until their summer growth was about completed, and that the simultaneous destruction of feeding rootlets cut off the flow of sap suddenly. The fact that trees were stunted was an indication that the injurious effects of feeding by the aphides were felt earlier in the season.

In addition to trees noticeably stunted and others prematurely defoliated are found still others which show no external evidence of infestation and yet upon examination prove to be heavily infested. This phenomenon is frequently noticeable among young trees or in nursery rows, and hints at a power of resistance.

In orchards and districts where conditions favor large productions of winged forms, or migrants, spring and early summer infestations are small, denoting that few insects passed the winter on the roots. After the month of June, however, such infestations multiply rapidly and become very large by September, the month in which the fall migrants are produced in greatest abundance. After September there remain small wingless colonies which increase but little until the summer following. The winged forms are produced in abundance on heavy dry clay soils which crack in summer and autumn. Irrigated orchards produce them in smaller numbers than those that receive no moisture from May to October. On loam, silt, and light-clay soils the winged forms are much less abundantly produced. On such soils the infestation remains largely or wholly wingless the year around, and the conditions are generally unfavorable to such heavy infestations as occur on the heavy clays. The aphides appear to lack freedom of movement, and frequently their colonies are unable to increase perceptibly through summer. Occasionally the wingless infestations are severe the year round; where this is so, in the early part of the year there is caused a considerable stunting of growth and more or less

weakening, unless the trees can put out plenty of new rootlets to replace those injured and destroyed. This condition has been noted especially on light-clay soils where poor cultivation was employed.

## SPREAD OF THE INSECT

In nurseries under favorable conditions the spread of the insect may be rapid. A half-acre pear nursery examined on June 9, 1915, failed to show infestation, though the aphis was probably present. When visited four months later, on October 16, it was found that more than half the trees examined were infested, some quite heavily. In large orchards where the soil is permeated throughout with rootlets the aphis doubtless is very easily diffused through the soil. In young orchards conditions indicate that not much spread takes place from tree to tree. Infested young orchards generally point to the nursery as the source of infestation, but the possibility of infestation through the winged forms, or migrants, must be considered. A knowledge of the full life cycle of the insect alone can clear up this point.

## BIOLOGY AND DESCRIPTION OF THE INSECT

The wingless individuals live chiefly on the small rootlets and less frequently on roots and the underground portions of the sucker growth. They are always somewhat elongate and are for the most part pale yellowish red, but they may vary from a pale pink or yellow to deep red. They are rather sparsely clothed with long, curling, woolly, or cottony filaments, of which there are four or six on each segment. Toward the end of each instar these filaments are longer than the body—often three times as long. There is a sparse whitish powder on the body, more abundant at the caudal end. The cornicles appear as dusky-rimmed pores. The young are pale yellowish red and elongate.

The pupæ develop on the same portions of the tree as the wingless forms. They are very elongate in form and are clothed as are the wingless. The wing pads are inconspicuous and are white or light gray. As a rule pupæ on a rootlet develop almost simultaneously. The winged forms issue together, after which the narrow, elongate, cast pupal skins are conspicuous in little heaps, and are easily distinguishable from those of the wingless forms.

In the Walnut Creek district pupæ and winged migrants were collected in appreciable numbers from August 25 to November 17, and as late as December 22 a nymph was found. These forms were most abundant in September, and this observation apparently holds true for other localities in California. Wingless colonies collected at San Jose, Cal., on June 10 and thereafter, kept in Petri dishes with moist sand in a cellar, produced pupæ on July 20 and migrants from July 24 to August 7. This appeared to be abnormally early in the year for the production of winged forms, and

it may be that the environment and conditions hastened it. Under favorable conditions of soil the migrants were produced in great abundance on both young and old pear trees. In many cases, especially on young trees, it appeared that fully 90 per cent of the aphides observed at one time were pupæ, and in other instances observations in October and later after the winged forms had departed indicated that almost the entire infestation had developed into migrants. On old trees there remained on the average a larger residue of wingless forms. On unfavorable types of soil the winged forms are produced in far less abundance. It appears to be a rule that the heavier and drier the soil the larger the percentage of pupæ developing. It sometimes happens that the migrants are unable to rise to the surface of the ground and become imprisoned in pockets in the soil. In one instance two living sexual females were found in such a pocket beside dead migrants.

The winged forms have been noticed on pear foliage and on the trunk, but with one exception [1] no deposition of sexes has been observed on the pear. On cork and American elms (*Ulmus* spp.) migrants were observed to deposit the sexes in cracks in the bark and on the lower surface of leaves. In one instance the migration from a nursery of pear trees to a group of young elms 200 yards distant could be traced. The migrants fly readily and strongly and are stimulated by the sun's rays, being more active on warm than on cool days. On the elms they were more abundant on trees with rough bark than on the smooth-barked plants.

The migrants vary considerably in size. They are rather elongate, shining black or dark green, with a tuft of white wool on the caudal segment; otherwise, there is no flocculence. The lower surface is dark green, sparsely powdered at the sutures. The antennæ, eyes, and a portion of the legs are black. The base of the femora and the middle portion of the tibiæ are yellowish brown or amber. The wings have narrow black veins and a greenish blue stigma. The wing insertions are sometimes brown, but are more often yellowish. In recently molted individuals there is sometimes a smoky-brown patch at the base of the fore wings.

To obtain the sexes, migrants were confined in stender dishes and in small rubber cells mounted on microscope slides with cover glasses as lids. Some were kept in a lighted room in which the temperature varied very considerably, at times rising to 75° and at other times falling to 55° F. Others were kept in a dark cellar where the temperature varied but little and averaged about 61° F. Under cellar conditions the migrants deposited more sexual forms than under the conditions obtaining in the room. Some of the dishes were kept dry and others moistened to different degrees. In the moistened dishes the sex pro-

[1] In August, 1911, at San Jose, Cal., a migrant was noticed depositing sexes on the upper surface of a pear leaf.

duction was better than in the dry ones, although too much moisture prevented the sexual forms from freeing themselves from the pellicles. Whether the migrants had flown or not did not seem to bear any influence on the deposition of the sexual forms. In most of the dishes more than half of the sexed forms were not extruded, but died unborn. In the rubber cells five-eighths of an inch in diameter and three-sixteenths of an inch in height the migrants did best singly, while the larger stender dishes provided space for a number. In all the dishes pieces of pear or elm bark were provided, but the migrants rarely deposited the sexes on these, nearly always extruding them on the filter paper also provided. It frequently happened that the sexes after having been extruded became entangled with the wings or legs of the parents or with each other. The sexes were deposited in rapid succession. The migrants rarely lived beyond three days after they were placed in the dishes, whether they deposited sexual forms or not. None lived longer than six days. They died immediately after the sexes had been extruded and very few deposited their full complement.

All the sexes deposited were not noted; but about four-fifths of them totaled 109 individuals, of which a little over half (58) were females. Only a few matured, and the majority died unmolted. Undoubtedly the cause of this was the abnormal condition of the environment. However, it appears to be proved that the sexes are produced in about equal numbers, and observations in the field corroborate this. Four fall migrants dissected on October 27 and 28 had contained, respectively, 5, 7, 8, and 9 young. In the dishes not more than seven sexes were ever dropped by an individual. The number of males and females deposited by individual migrants was found to range from seven females and no males to five males and one female. Probably a larger series would have furnished a migrant producing only males. As a rule the production of sexes was about evenly divided between male and female.

The sexes have no woolly covering such as that occurring on the sexes of *Eriosoma lanigerum*, but are bare and shining. The female, however, at the time of depositing the winter egg, has a patch of short white wool on either side of her body and with this she contrives to clothe partly the winter or impregnated egg. The sexes are active, the male especially so, both immediately after extrusion and following the casting of their fourth and final skin. Between casting their first and fourth skins they remain inactive unless disturbed. Normally they seek crevices in the bark, but in the dishes they frequently molted on filter paper or on the sides and floor.

The sexes mature in from 7 to 11 days and molt four times—that is, about every other day. Being beakless, they take no food.

The males are smaller than the females, the latter being enlarged by reason of the egg within the body. The male at first is light green, with

hyalin antennæ and legs and black eyes of three facets. The insect becomes darker with age and the mature individual is dark olive-green, sometimes tinted with lilac or purple, the central part of the abdomen being darkest. The male is always narrow in shape. The female varies in color from a light orange to a dark red. The eyes and appendages are as in the male. The majority are orange or a light crimson-lake. They are much stouter than the males and are longer and stand much higher. A mature female measured alive was 0.67 mm. long by 0.33 mm. in maximum width. A mature male was 0.43 mm. long by 0.21 mm. in maximum width.

Copulation occurs as soon as the sexes are mature. It appears that unless the female is fertilized directly after she has cast her last skin she will fail to deposit the winter egg. The male may live at least a week after he is mature, but apparently he can exercise the sexual function only immediately after he has cast the last skin. The females deposit the impregnated egg immediately after copulation, and after its deposition they may live for a day or two at the most. The winter or impregnated egg is laid normally in crevices or scars of the bark of the elm. In the dishes it was laid sometimes on the outside of the bark, and both elm and pear bark were used. It was never laid elsewhere than in the bark. The egg measures about 0.444 mm. by 0.225 mm., is short oval, reddish yellow, and shining. The end first extruded is reddish and bare, while the other extremity is yellowish and usually covered with short white wool provided by the female. Winter eggs were deposited in dishes between October 15 and November 12. Undoubtedly they occur in nature as early as September 5, and may be laid as late as the middle of November. Toward the end of October some were collected under the bark of elms under observation. Table I is a comparison of the biology of *Eriosoma pyricola* with that of *E. lanigerum*.

TABLE I.—*Comparison of biology of Eriosoma pyricola with that of Eriosoma lanigerum in California*[1]

| *Eriosoma lanigerum* on apple and varieties of pear. | *Eriosoma pyricola* on pear. |
|---|---|
| Aerial and radical. | Radical only. |
| Attacks trunks, branches, and twigs; causes knotty swellings on roots. | Attacks chiefly fibrous rootlets; rarely causes lesions; occasionally settles on larger roots. |
| Fall migrants rarely abundant; apparently not influenced by conditions. | Fall migrants very abundant under favorable conditions. |

[1] The full cycle of these species has not been worked out in California, but there appear to be no records of spring generations of *E. lanigerum* observed on elm.

The fall migrants of *E. pyricola* may be distinguished from those of *E. lanigerum* and *E. americanum* as shown in Table II.

TABLE II.—*Comparison of the fall migrants of Eriosoma pyricola, E. lanigerum, and E. americanum*

| *E. pyricola.* | *E. lanigerum.* | *E. americanum.* |
|---|---|---|
| Stigma short, greenish blue. | Stigma somewhat elongate, yellowish or gray. | Stigma elongate, gray. |
| Veins narrow without brown margins. | Veins narrow and without brown margins. | Veins broad, with brownish margins. |
| Body naked except for caudal segment. | Body with some woolly clothing. | Body with slight woolly covering. |
| Distal sensoria of antennal segments V and VI with fringe. | Distal sensoria of antennal segments V and VI without fringe. | Distal sensoria of antennal segments V and VI without fringe. |

The new species is easily distinguished from *E. ulmi* Linnæus from the fact that segment V bears prominent transverse sensoria. The wingless forms can be distinguished from those of *E. lanigerum* by the structure of the compound wax pores, and the winged forms by the antennæ. The winged forms of *E. pyricola* are remarkably like those of *E. lanuginosa* Hartig. The proportions are almost exactly the same. The only difference seems to be the fringing of the sensorium on segment V. The wingless forms and the pupæ have the prominent wax pores figured. No such pores occur in our specimens of *E. lanuginosa*, but very similar ones do occur in *E. ulmi*. At first it was thought that two species were present in the collected material, but careful rearing experiments by the junior writer have shown the connection between all the forms. It does not seem probable that such prominent wax-secreting structures would be present in one form of the species and not in all forms.

**Eriosoma pyricola, n. sp.**

WINGLESS VIVIPAROUS FEMALE.—General form elongate. Antennal segments in length as follows: I, 0.048 mm.; II, 0.048 mm.; III, 0.1 mm.; IV, 0.04 mm.; V, 0.048 mm.; VI, 0.064 mm. (unguis, 0.032 mm.); segments armed with hairs (fig. 1, *E*), which are considerably longer than those met with in *lanigerum* (fig. 1, *D*), and with a large distal fringed sensorium on segments V and VI, as well as some smaller ones on VI. Compound wax pores very prominent and circular (fig. 1, *I*), those on the abdomen containing about 20 cells. Abdomen sparsely covered with hairs about 0.16 mm. long; cornicles circular, their rims more heavily chitinized on their inner margins than elsewhere. Wax reservoir apparently present as in *E. lanigerum* (visible as a clear yellow area in mounted specimens). Hind tibiæ about 0.44 mm. long; hind tarsus, 0.112 mm.; rostrum extending beyond the second pair of coxæ. Length, 1.92 mm.; width, 0.96 mm. The hairs on the antennæ of the young are especially prominent (fig. 1, *J*).

Young forms yellowish pink, older ones pink to red. Antennæ, legs, and labium dusky; eyes dark red, very minute.

INTERMEDIATES.—In the collection, Q. 6399, are a number of specimens which would be taken at first glance for wingless viviparous females. A careful study, however, proves them to be intermediates. No trace of wing pads can be found, but the eyes clearly show the intermediate nature of the specimens. In the normal wingless

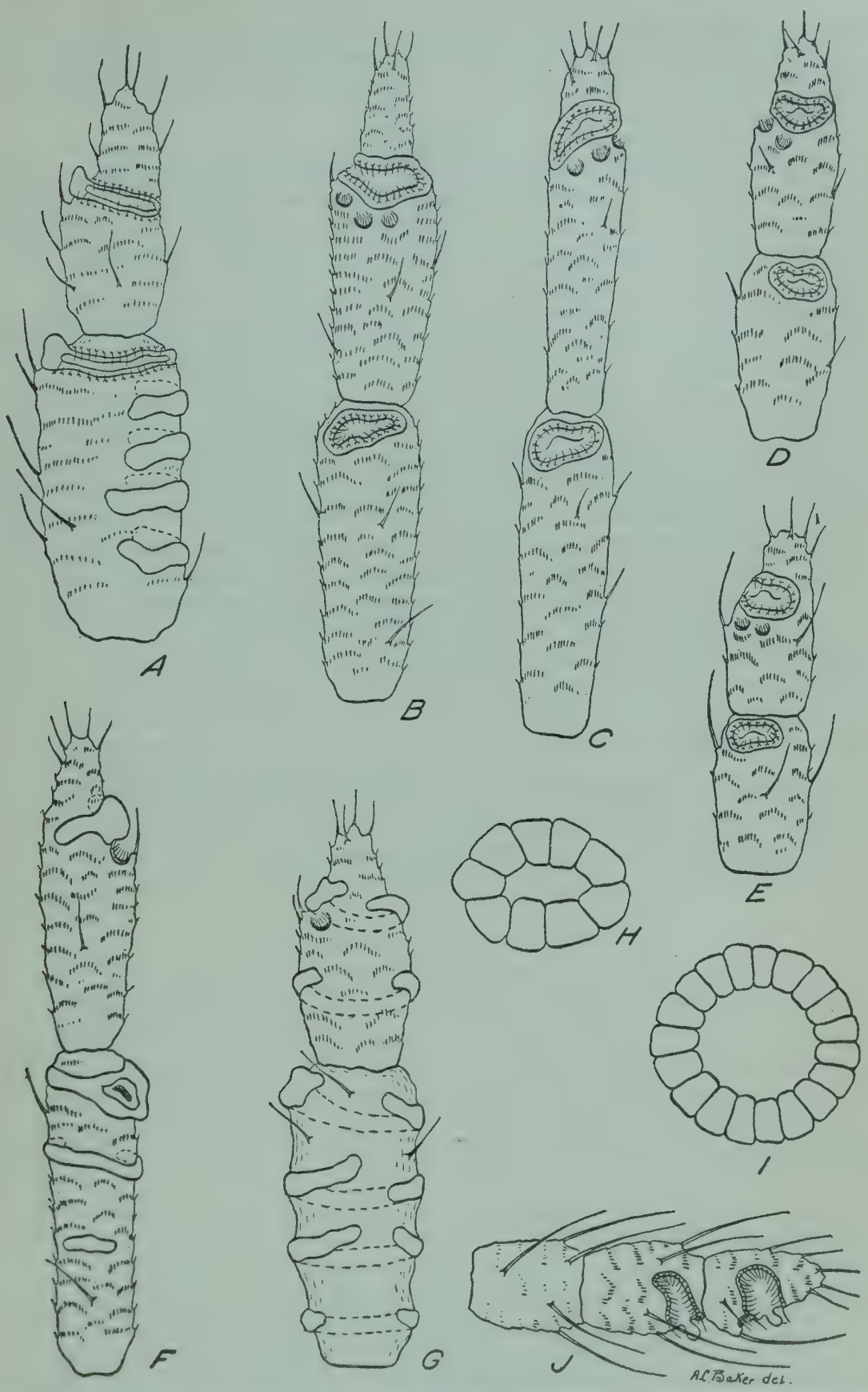


FIG. 1.—Comparative structure of antennæ and wax pores of *Eriosoma* spp.: *A*, distal segments of antenna of winged viviparous female of *E. pyricola*; *B*, distal segments of antenna of winged viviparous female of *E. ulmi*; *C*, distal segments of antenna of wingless viviparous female of *E. americanum*; *D*, distal segments of antenna of wingless viviparous female of *E. lanigerum*; *E*, distal segments of antenna of wingless viviparous female of *E. pyricola*; *F*, distal segments of antenna of winged viviparous female of *E. americanum*; *G*, distal segments of antenna of winged viviparous female of *E. lanigerum*; *H*, compound wax pore of *E. lanigerum*; *I*, compound wax pore of *E. pyricola*; *J*, distal segments of antenna of first instar wingless viviparous female of *E. pyricola*.

forms the eyes are composed of three facets and are very minute, whereas in these specimens the eyes are large and composed of numerous facets, thus approaching the compound eyes of the winged form. All other characters met with are those of the wingless viviparous female.

PUPA.—Antennal segments in length as follows: I, 0.048 mm.; II, 0.064 mm.; III, 0.192 mm.; IV, 0.064 mm.; V, 0.08 mm.; VI, 0.08 mm.; segments armed with hairs and sensoria as in the wingless female. Wing pads about 0.64 mm. long. Compound wax pores similar to those of the wingless females. Hind tibia, 0.432 mm.; hind tarsus, 0.128 mm. Body with long hairs as in the wingless form. Length, 2.32 mm.; width, 0.96 mm.

Pinkish, with a brick-red diffusion; wing pads whitish yellow; wool sparse, erect.

WINGED VIVIPAROUS FEMALE (FALL MIGRANT).—Antennal segments in length as follows: I, 0.048 mm.; II, 0.064 mm.; III, 0.432 mm.; IV, 0.112 mm.; V, 0.112 mm.; VI, 0.08 mm. (unguis, 0.032 mm.); segments I and II armed with a few hairs; segment III armed with about 20 transverse sensoria, which extend a little over halfway around the segment as in *E. lanigerum*, the dorsal side of the segment armed with numerous prominent hairs; segment IV similar to segment III and armed with four or five transverse sensoria; segment V (fig. 1, *A*) armed with three or four transverse sensoria and a distal fringed sensorium, a few hairs, and many rows of setæ; segment VI similar to segment V, but without transverse sensoria. The fringed sensorium at the base of the unguis varies in shape. Forewing somewhat similar to that of *E. americanum;* stigma short and rounded at the distal extremity. Hind tibia, 0.88 mm.; hind tarsus, 0.128 mm. Form elongate; length, 1.76 mm.; width, 0.72 mm.; forewing, 2.4 by 0.88 mm. Without wool.

Dark brown or very dark green. Base of femora and tibiæ yellowish gray. Stigma bluish gray. Abdomen shining.

Described from wingless females, intermediates, pupæ, and winged viviparous females in balsam mounts.

*Type:* Cat. No. 20083, U. S. National Museum.

# PATHOLOGICAL HISTOLOGY OF STRAWBERRIES AFFECTED BY SPECIES OF BOTRYTIS AND RHIZOPUS

By Neil E. Stevens,
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## INTRODUCTION

The fungi causing rots of strawberries (*Fragaria* spp.) in transit from the Southern States have been under investigation by Dr. C. L. Shear, Mr. R. B. Wilcox, and the writer for the past two years. From the first it has been apparent that two species were chiefly responsible for their decay during shipment and on the market. These were *Botrytis* (*cinerea*?) and *Rhizopus* (*nigricans*?).[1] The effect of these two fungi on ripe strawberries is strikingly different. Berries injured by *Botrytis* sp. show a characteristic dryrot—that is, they retain their shape, shrivel somewhat, and no leaking of juice is evident; whereas berries rotted by *Rhizopus* sp. quickly flatten out, with the loss of a large amount of juice. Such berries are characterized as "leaks" by growers and dealers.

F. L. Stevens[2] has already recognized a species of Rhizopus as the probable cause of leak. He, however, considers (p. 950) that *Botrytis* sp. "is the primary cause of the molding, that the *Botrytis* initiates the decay, opening the way to such other saprophytes as may be present; of such saprophytes, *Rhizopus* is by far the most prominent and most abundant." In order to determine if possible the relations of these fungi in rotting strawberries and in particular what differences exist in their method of attack on the fruit, a study of strawberries affected by these fungi was undertaken.

## EXPERIMENTAL METHODS

The strawberries examined were chiefly of the Klondike variety grown in Louisiana during the season of 1915. Berries of other varieties grown in South Carolina and at Arlington Experimental Farm, Va., in 1915, as well as the Missionary and Klondike varieties from Florida in 1916, were used for comparison. Naturally infected berries as well as sound berries inoculated with spores and mycelium from pure cultures were used in both cases.

The material was fixed in a solution of equal parts of absolute alcohol and glacial acetic acid. This fluid penetrates very rapidly, so that whole strawberries are satisfactorily fixed. In the case of large berries,

[1] In the present uncertainty regarding the taxonomy of these genera it seems unwise to attempt a definite determination of the species. Permanent mounts of the material described are preserved, however, and cultures of the species considered are retained for further study.

[2] Stevens, F. L. A destructive strawberry disease. *In* Science, n. s., v. 39, no. 1017, p. 949–950. 1914.

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however, the ends were cut off to hasten penetration. Strawberry cells are so large that rather thick sections, from 10 to 20$\mu$, were found most desirable. The walls of the strawberry cells and of the fungus hyphæ are so similar that differential staining was rather difficult. The best differentiation was secured by a combination of methylene blue and clove-oil eosin, using a water solution of tannin as a mordant. This method was suggested to the writer by Mr. Charles S. Ridgeway, of the Bureau of Plant Industry. The hyphæ, however, are so large as to be easily distinguished when the sections are properly stained with the more permanent stains, as safranin, Delafield's hematoxylon, or even Bismarck brown.

## RESULTS OF INFECTION OF STRAWBERRIES BY BOTRYTIS SP.

*Botrytis* sp. has long been a favorite subject for the investigation of the relations of host and parasite. The somewhat conflicting views held by different investigators as to the nature of its attack on the host are well summarized by Brown [1] in a recent paper. In general, all writers agree on the presence of a cell-wall dissolving enzym, but differ widely as to the cause of the toxic action of the fungus.

As already stated, strawberries rotted by *Botrytis* sp. retain their shape, shrivel slightly, and even in a moist chamber there is no evident leaking. The moisture is apparently lost so slowly that it evaporates from the surface of the berry. A microscopic examination shows that the fungus has penetrated all parts of the berry; indeed, the cells are in many places embedded in the mass of mycelium and are apparently held together by it. The fungus is evidently capable of readily dissolving the middle lamella and of penetrating the cell walls themselves. Often hyphæ grow between the cells of the host for some distance and then penetrate the cells (Pl. XLIX, *A*). Not infrequently cells containing numerous hyphæ have the shrunken and distorted protoplasmic contents still present (Pl. XLIX, *B*, *C*, *D*). Sometimes hyphæ occur in adjacent cells whose separating wall remains intact and apparently unchanged (Pl. XLIX, B); or they may pass from one cell into the next, either where the cells are in contact or across an intercellular space (Pl. XLIX).

It is interesting to observe that hyphæ usually enter a cell at the angle where it joins two other cells; Plate XLIX, *D*, *F*, and *G*, shows examples. The hypha passes between two cells, apparently by dissolving the middle lamella, and then penetrates the wall of the cell with which it comes in direct contact. Occasionally a hypha seems to push back a portion of the cell wall before penetrating (Pl. XLIX, *G*). The fungus may, however, penetrate the wall at a considerable distance from the intersection of the cells (Pl. XLIX, *A*, *E*); or it may

[1] Brown, William. Studies in the physiology of parasitism. I. The action of Botrytis cinerea. *In* Ann. Bot., v. 29, no. 115, p. 313–348. 1915.

pass the point of intersection and penetrate a short distance beyond (Pl. XLIX, *H*).

Brown,[1] working with thin disks of tissue cut from various plants, particularly tubers of the potato and roots of the turnip, immersed in a strong extract from the germ tubes of *Botrytis cinerea*, noted that the separation of the cells followed the line of the cell walls, the cells on either side being left intact. His idea of the destruction of the cells is that the middle lamella is first dissolved, in consequence of which the tissue readily falls apart along the line of the middle lamella. Very soon the remainder of the cell wall disintegrates and the whole structure becomes very fragile.[2] In no case was complete solution of the cell wall observed. Death of the cells [3] takes place at a late phase in the process of disorganization of the cell walls. He observed also that in all cases if a cell wall was disintegrated death of the cell ensued; on the other hand, if the cell wall was not affected neither were the living contents of the cell.[4]

Brown's conclusions satisfactorily explain the condition found by the writer in strawberry cells attacked by *Botrytis* sp. Certainly the fungus is able to penetrate the cells of the host while they are still fairly normal in appearance and while the cytoplasm is still distinguishable (Pl. XLIX, *B*, *D*, *G*). The writer did not find, however, in any of the strawberries examined cells which were unaffected by the action of the fungus.

## RESULTS OF INFECTION OF STRAWBERRIES BY RHIZOPUS SP.

In contrast to the condition of strawberries rotted by *Botrytis* sp., berries rotted by *Rhizopus* sp. show the following characteristics. The berries soon become flattened, with considerable loss of juice. Microscopic examination shows that the hyphæ are characteristically close to the surface of the berry, the majority being found in the outer six or eight cell layers. Hyphæ rarely or never penetrate the cells of the berry under field conditions or when kept in moist chamber. The nuclei of the cells persist in apparently normal condition until the cytoplasm of the cell has almost entirely collapsed.

The crowding of the fungus in the outer portion of the berry is very noticeable. Indeed hyphæ frequently grow for some distance immediately beneath the epidermis. Plate XLIX, *I*, shows a portion of such a hypha in a section cut nearly tangential to the surface of the berry. The small, thick-walled cells (heavy lines) on the right are epidermal cells; the larger, thin-walled cells (light lines) on the left are storage cells. The hypha, which could be traced across several sections, grows between these two layers of cells for a considerable distance without penetrating either. A similar condition is shown in vertical section in Plate XLIX,

[1] Brown, William. Op. cit., p. 333.
[2] Ibid., p. 335.
[3] Ibid., p. 347.
[4] Ibid., p. 345.

*K, L.* In the latter case the fungus has penetrated the epidermis and the external hyphæ are sporangiophores.

It is evident from a study of the sections that *Rhizopus* sp. does not readily penetrate the unbroken epidermis from the outside. Hyphæ are found which extend for some distance along the surface of the berry without penetrating. Plate XLIX, *J*, shows a portion of such a hypha; even the germ tubes seem unable to penetrate readily and often grow for some distance (Pl. XLIX, *M*) over the surface without penetrating.

Under field conditions or in moist chamber in the laboratory *Rhizopus* sp. apparently very rarely enters the host cells. Although several hundred slides were examined no single instance was found in which a hypha had penetrated a cell wall. Plate XLIX, *I–L*, shows that the hyphæ typically grow between the cells along the middle lamella. The effect of the fungus on the host cells is readily seen by the contraction of the protoplasm. Plate L shows strawberry cells in various stages of degeneration close to hyphæ of *Rhizopus* sp.

Plate L, *A*, shows the normal appearance of one of the smaller storage cells of the strawberry. In this case the cytoplasm contains numerous small vacuoles. Frequently, especially in larger cells, there is a single large vacuole. Plate L, *B*, shows a similar cell in which the protoplasm has begun to contract away from the wall. This cell was separated from the nearest hyphæ by three layers of cells. In Plate L, *C*, hyphæ of *Rhizopus* sp. are shown in contact with two host cells (a branch hypha overlies one cell). The protoplasm of these cells is much shrunken, but the cell walls retain their normal position, and the nuclei are unchanged. Plate L, *D*, *E*, *F*, and *G*, show progressively later stages in the breaking down of cells adjoining hyphæ. In some (Pl. L, *D*, *F*) the wall has begun to collapse. In all except Plate XLIX, *G*, in which there was very little cytoplasm remaining, the nucleus shows no signs of degeneration.

This persistence of the nucleus in apparently normal condition after the contraction of the protoplasm has progressed considerably is one of the most striking characteristics in berries attacked by *Rhizopus* sp. and is in sharp contrast to the condition found in berries rotted by *Botrytis* sp. Often in a cell in which the cytoplasm has largely disappeared and the wall is partly collapsed the nucleus appears large and typical, as in an intact cell (Pl. L, *J*). Frequently the cell wall collapses so rapidly that no space is left between it and the contracted protoplasm (Pl. L, *H*, *I*).

## EFFECT OF RHIZOPUS SP. ON BERRIES IN EXTREMELY DRY AIR

In connection with experiments on the humidity relations of the fungus, berries inoculated with *Rhizopus* sp. were placed in a desiccator with concentrated sulphuric acid. Under these extremely dry conditions the berry "leaked" in the characteristic manner, but the habit of growth of the fungus was changed in two important particulars.

Fungus hyphæ were found in all parts of the berry, being abundant even in the center, within the circle of vascular bundles. Apparently the extreme dryness of the surrounding air made the intercellular spaces within the berry more favorable for its growth than the outer ones. Under these severe conditions the cells of the berry collapsed so generally that the relations of the fungus hyphæ to the walls could usually be studied only in cells near vascular bundles. It was evident that while, in general, the hyphæ grew between the cells of the host (Pl. L, *L*) they were frequently found inside the cells as well (Pl. L, *K*, *M*). It is worthy of note that in these berries several instances were found where hyphæ had punctured the cells and the nucleus of the cell was unchanged in appearance (Pl. XLIX, *K*).

## COMPARISON OF THE FUNGI

The difference in the histological relations of the two fungi with the strawberry may be briefly summarized as follows:

*Botrytis* sp. penetrates all parts of the berry, growing within the cells as well as between them and ramifies through the tissues of the strawberry, surrounding and filling them with a network of mycelium. The cells of the berry seem to be quickly killed by the fungus; at least the protoplasm shrinks away from the cell wall and becomes disorganized so that no nucleus can be distinguished.

The mycelium of *Rhizopus* sp., on the other hand, is found chiefly in the outer portion of the berry. The hyphæ grow between the cells, separating them and apparently extracting the cell sap. The nuclei of the cells persist unchanged until a late stage in the breaking down of the cytoplasm. When the fungus is grown on berries in a dry atmosphere, its action is somewhat different. The mycelium penetrates to the center of the berry, and hyphæ are frequently found inside cells.

It is difficult to trace an exact causal relation between the histological differences in the attack of these fungi on the strawberry and the fact that they cause quite different types of rot. The fact that *Rhizopus* sp. separates the cells of the berries so completely may readily account for the berries affected with this fungus becoming so soft and easily flattened. On the other hand, the mycelium of *Botrytis* sp., by penetrating all parts of the strawberry, helps to hold it in shape and converts it into a mummy. It is possible that the juice of the berries affected by *Rhizopus* sp. is pressed out by the collapse of the berries, owing to the mere separation of the cells. This is, however, hardly an adequate explanation of the phenomenon.

While it is not proposed at the present time to review the rather voluminous literature on either of the fungi under consideration, a closely parallel case described by Behrens [1] should be mentioned in this

[1] Behrens, Johannes. Beiträge zur Kenntnis der Obstfäulnis. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 4, No. 12, p. 515–516. 1898.

connection. He observed in 1895 ripe tomatoes affected by *Mucor stolonifer* which reduced the pulp of the tomato to an almost fluid mass. A species of Fusisporium found at the same time on the tomatoes produced a dry-rot quite in contrast to the wet condition produced by the species of Mucor. Behrens found on microscopic examination that the mycelium of *Fusisporium* sp. penetrated the cells of the host, while the mycelium of *Mucor stolonifer* grew entirely in the intercellular spaces.

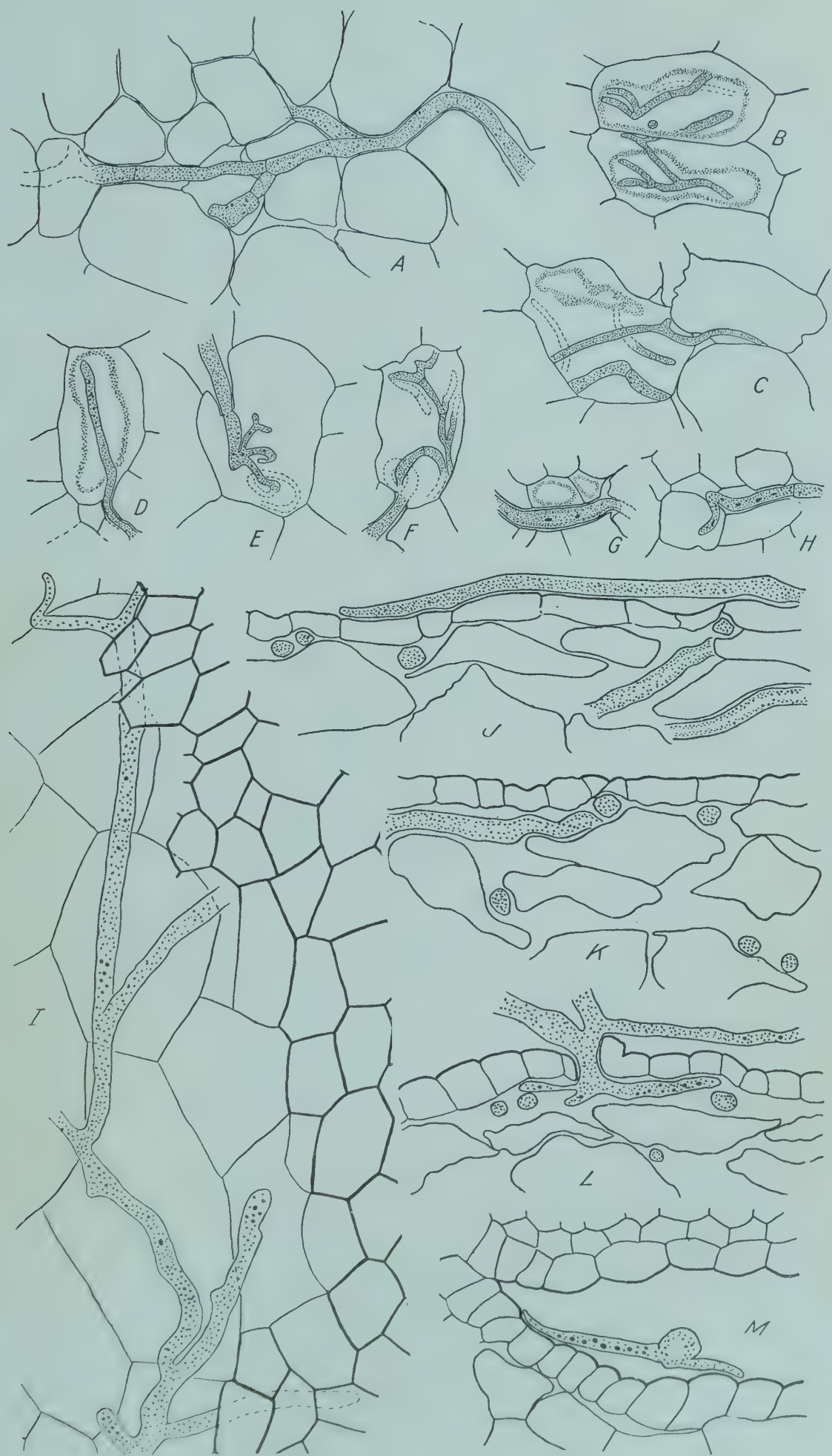
The relation of these fungi to each other in their attack on the berry is much clearer. In comparatively few cases have both fungi been found on the same berry and in no instance has the writer found a berry in which *Rhizopus* sp. had followed in a place originally infected by *Botrytis* sp.

Numerous cases have, of course, been found in which there were two fungi in the same berry; for instance, *Botrytis* sp. and *Fusarium* sp., *Botrytis* sp. and *Alternaria* sp., *Rhizopus* sp. and *Fusarium* sp. These fungi do not, however, seem to have entered in the same place, but rather from different portions of the berry. The mycelia of the two fungi sometimes mingle in the tissues of the berry—for example, *Botrytis* sp. and *Fusarium* sp., *Rhizopus* sp. and *Fusarium* sp.—or they may occupy different portions of the berry with a marked line of division between them, each apparently being unable to invade tissue occupied by the other fungus—for example, *Botrytis* sp. and *Alternaria* sp.

These observations do not preclude the possibility of *Rhizopus* sp. following in an area originally infected by *Botrytis* sp. or some other fungus, and this may occur in the field or in badly affected berries which are thrown out as culls in packing. They do, however, plainly indicate that *Rhizopus* sp. is not dependent on the presence of any other fungus in its attack on strawberries during shipment and on the market.

## PLATE XLIX

*A–H*, Strawberry cells attacked by *Botrytis* sp. (× 210): *A*, Hypha growing partly between and partly within strawberry cells; *B*, hyphæ inside strawberry cells in which remnants of the protoplasm may still be distinguished; *C*, hypha passing from one cell into another across a short intercellular space; *D*, *E*, *F*, *G*, *H*, hyphæ entering cells in various ways (in *G* the hypha has pushed back a portion of the cell wall before breaking through). *I–M*, Strawberry cells attacked by *Rhizopus* sp. (× 210): *I*, Hypha growing between the epidermis and the adjacent layer of storage cells; *J*, hypha growing over the surface of the strawberry; *K*, hyphæ growing underneath the epidermal layer and between the storage cells; *L*, *Rhizopus* sp. growing between epidermal cells (basal portions of sporangiophores above and rhizoids below epidermis); *M*, germinating spore in cavity formed by a seed.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## PLATE L

Strawberry cells attacked by *Rhizopus* sp. *A*, Normal storage cell of strawberry; *B*, storage cell (near hyphæ) showing a slight contraction of the protoplasm; *C*, *D*, *E*, *F*, *G*, progressive contraction of protoplasm of host cells near hyphæ (the cell walls have contracted very little); *H*, *I*, *J*, strawberry cells near hyphæ in which the cell wall has crumpled with the contraction of the protoplasm; *K*, *M*, hyphæ inside cells; *L*, hyphæ growing between cells of the strawberry; *K*, *L*, *M* are drawn from berries which had been rotted in the desiccator. (× 210.)

# LIFE HISTORIES AND METHODS OF REARING HESSIAN-FLY PARASITES

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## INTRODUCTION

The most effective factors in the control of the Hessian fly (*Mayetiola destructor* Say) in the past have been its parasites. There are seasons, however, when the parasites become scarce and the Hessian fly exceedingly abundant. Again, in the same season the Hessian fly seems practically free from parasites in some localities while in others its parasites are numerous. A thorough knowledge of the life histories, field habits, relative efficiency, and effective methods of artificial propagation and dissemination of the different parasites, therefore, might make it practicable to introduce the most efficient species from localities where they are abundant into other localities where the host is working destruction unchecked by its enemies. It might also be possible to propagate artificially and to disseminate the parasites during periods when they have become scarce in the fields, and thereby shorten the period of destructive abundance of the Hessian fly. Up to the present time very little accurate and detailed information seems to have been recorded regarding the life stages, habits, and efficiency of Hessian-fly parasites. It has been uncer tain whether or not some of the species involved were true parasites. Some results in this direction have been accomplished by the author during the last two seasons, and the purpose of this paper is to make public these results and the methods used in attaining them.

The life histories and methods of rearing three hymenopterous parasites are treated in this paper: *Eupelmus allynii* French, *Merisus destructor* Say, and (*Merisus*) *Micromelus subapterus* Riley. The seasonal history and field habits of these parasites will require another season's observation before they can be effectively treated. The scope of this paper is therefore limited to the life histories and relationships of these species to one another and to their common host as determined under laboratory conditions.

[1] The writer wishes to acknowledge his indebtedness to Messrs. E. O. G. Kelly, W. R. Walton, A. B. Gahan, W. R. McConnell, and J. A. Hyslop, all of the Bureau of Entomology, for helpful advice; to Mr. Kelly for making the work possible, and to Mr. Gahan for determining all specimens.

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## METHODS OF BREEDING AND REARING

The adult parasites used in all experiments were kept in modified forms of the Doten cage.[1] One form, used when it was desired to confine a number of parasites together, consisted of two large, straight-sided vials of the same diameter, the mouths of which snugly fitted into a paper tube 1 inch long. This paper tube was held in shape by a layer of adhesive plaster around the outside. The cage was prevented from rolling by sticking a square of heavy cardboard to one side of the connecting tube. A label was pasted to the upper side of the tube for identification. One vial was kept dry and clean, while water and honey were supplied in the other.

The other form of Doten cage, used chiefly for isolating pairs and individuals, was simply a small, straight-sided vial into the mouth of which was fitted the open end of a slightly smaller, straight-sided vial. A small label was pasted on the side of the larger vial for identification. Cages of this kind were prevented from rolling by keeping them in shallow boxes with corrugated pasteboard-lined bottoms. Food and water were placed in the smaller vial.

In both forms of cages the water and the honey used for food were placed separately in small droplets on the upper surface inside the food vial. The honey used was the extracted form diluted with an equal amount of water. It was necessary to exercise considerable care not to place too large a drop of honey in a cage, because of its tendency to run down on the inside of the vial and to entangle the insects. Fresh water and honey were placed in the cages daily, and at least once a week the food vials of the cages were carefully cleaned to remove dried or soured honey. Replenishing the food and water in the cages once a day seemed sufficient to supply the needs of the parasites. It was often found necessary to make up a fresh supply of the honey because of souring or molding, especially in hot weather. Sterilizing the fresh supply by placing the dropper bottle containing it in boiling water for a few minutes caused it to remain sweet and usable much longer.

## BREEDING THE PARASITES

To determine all the life stages from egg to adult involved the processes of exposing Hessian-fly puparia to parasites, dissecting the parasite eggs from the host puparia, and rearing, in little glass-cell cages devised for the purpose, the resulting parasite larvæ on Hessian-fly larvæ which were also dissected from puparia. Hessian-fly puparia contained in sections of wheat stems were first exposed to the adult parasites by placing the stems in the vial cages containing the adults. The stems remained in the cage for a day, or until a parasite was seen to oviposit in a flaxseed, when they were removed and the puparia dissected. The

[1] Doten, S. B. Concerning the relation of food to reproductive activity and longevity in certain hymenopterous parasites. Nev. Agr. Expt. Sta. Tech. Bul. 78, 30 p., 10 pl. 1911.

eggs of the three species studied were always found between the inner surface of the puparium and the larva itself. They were transferred separately, each to an unparasitized Hessian-fly larva which had been previously dissected from its puparium and placed in a little glass-cell cage of the following description:

Flat glass plates 1 inch by 1¼ inches square were used, in which hollows about the size and shape of a Hessian-fly puparium were ground in one surface, one hollow per plate, this work being done with a small carborundum grinder. After a host larva and a parasite egg had been placed in a hollow, the cell was closed by covering it with an ordinary glass cover slip. The cover glass was held in place by two little dabs of honey on its underside. The cell was not sealed by a complete ring of the adhesive because of the desirability of diffusion of atmospheric moisture under the cover slip. Honey seemed to be the ideal adhesive for this purpose, since it had no odor harmful to the inmates of the cell; it held the cover-glass tight against the slide; it did not dry so hard as to prevent the cover-glass from being easily removed; and a supply of it was always convenient. A label was pasted on the glass plate near one end for identification. The complete development of the parasite from egg to adult on its host could then be observed under the binocular in this little cell without disturbing the parasite or the host in the least.

With each of the three species the period from oviposition to emergence of adult, when individuals were reared in glass cells, approximated very closely the period from egg to adult when individuals were reared under the same meteorological conditions in Hessian-fly flaxseeds. Hence, the length of each stage of development as determined from individuals reared in glass cells may be considered normal.

It was discovered that the larvæ of all three species molted while making their growth within the little cells. The length of the instars was not observed, but the number of molts was determined by transferring to a balsam mount on a microscope slide all the material left behind in the little glass cell where a single individual had made its growth. In cases where the larva had pupated, the last molted skin was added to the mount. In cases where the full-grown larva had not pupated, the mandibles borne by the larva were dissected from it and added to the mount. To determine the number of molts of a single individual, the mount of the material it left behind was examined under the microscope and the number of pairs of mandibles in the material ascertained. In all cases the cell in which the larva made its growth was known to be absolutely clean when the host and the egg from which the parasite larva hatched were placed in it; hence, it was known that all pairs of mandibles found in a mount belonged to the same larva. Cells which contained simultaneously the remains of more than one parasite larva were not used in determining the number of molts. No attempt was made to determine the number of molts of individuals which had made their growth inside flaxseeds.

## EUPELMUS ALLYNII

### THE EGG

The egg of *E. allynii* French (Pl. LI, fig. 1) is elliptical in shape, with a thin stalk of varying length on one or both ends. In some cases the stalk seems to be entirely absent from one end. The egg is grayish white in color. The long axis of the body of the egg averages 0.35 mm., the short axis 0.14 mm. in length. As a parasite of the Hessian fly, the observations at Wellington, Kans., indicate that the egg is normally deposited in the puparium of the host. Females were repeatedly observed by Mr. E. O. G. Kelly and the author to be very numerous in fields, ovipositing in Hessian-fly flaxseeds where these constituted the only stage of the fly to be found. In one instance, however, a wheat stem containing nearly grown Hessian-fly larvæ, but no flaxseeds, was placed in a vial cage containing females of *E. allynii*. Upon dissecting this stem two eggs of this parasite were found inside the leaf sheath close beside the Hessian-fly larvæ. Whether or not the parasite is able to complete its development on Hessian-fly larvæ before they have formed puparia is still unsettled.

Hundreds of flaxseeds in which *E. allynii* had oviposited have been dissected and the eggs of the parasite have always been found inside the puparium but external to the inclosed Hessian-fly larva or pupa. Sometimes they were unattached, but more often the egg was fastened to the inner surface of the puparium by a little netlike structure made apparently of fine, white threads tangled together (Pl. LI, fig. 2). The threads forming the net appeared to be identical in diameter, color, and material with the egg stalks. The edges of this little net or mat were fastened down all around the egg, holding it securely in place. Sometimes the net was partly fastened to the host larva in addition to the puparium. In all experiments *E. allynii* oviposited seemingly indiscriminately in flaxseeds already containing parasite larvæ as well as in those containing Hessian-fly larvæ. The incubation periods of 109 eggs varied from 1½ days to 5 days. The egg stage was shorter in summer temperatures, observations being made during a period from July to November.

### THE LARVA

Upon becoming fully formed inside the egg the larva (Pl. LII, fig. 2) breaks through one end of the chorion and after crawling around a little attaches itself to the external surface of the host larva. The parasite larva bears strong mandibles and feeds externally on the Hessian fly by puncturing the epidermis of the host and sucking out the body liquids. Larvæ reared in glass cells became full grown in from 7 to 10 days. After becoming full grown many of the larvæ were inactive for months; others pupated at once. In the warm summer temperatures most of the larvæ reared pupated at once upon completing their growth, while larvæ reared in the fall pupated only in occasional instances.

The larvæ reared in glass cells normally pass through five instars. Nearly all mounts made of the material left behind by larvæ which had finished feeding showed a total of five pairs of larval mandibles, while in the remaining mounts from two to four pairs were found which always correspond in size and shape to some one pair in the complete series. Five was the maximum number found in any one instance, and in cases where less than five were present it appeared that some of the molts had been lost in manipulation. Where five pairs of mandibles were found in a single mount, the sizes increased fairly uniformly from the second molt to the last. The mandibles and head shields of newly hatched larvæ appeared to be more heavily chitinized than those of later instars, except the last, and somewhat larger than those of the second instar. The mandibles of all instars are similar in shape. They articulate laterally with the head and fold together across the mouth, the ends overlapping. They are decidedly curved, taper to points, and are brown and chitinous. The sharp distal portions of the mandibles enlarge suddenly into a comparatively broad base bearing a chitinous lobe on the ventral side. The following average measurements will show the relative sizes of molted mandibles. These measurements represent the distance in a straight line, from the tip of the mandible to the shoulder, where the mandible suddenly enlarges into the broad basal portion.

| Molt No. | Length of mandible. |
|---|---|
| 1 | 0.016 mm. |
| 2 | .016 mm. |
| 3 | .024 mm. |
| 4 | .032 mm. |
| 5 | .048 mm. |

The full-grown larva is grayish white, averaging about 3 mm. long and 0.9 mm. in diameter, with 13 body segments besides the head. There are no tubercles on the head, but there is a row of four hairs evenly spaced across the top. The front of the head bears a pair of hairs, one on each side, just outside of each of which is a very short, white, conical projection, apparently antennæ. There is a short bristle near the base of each mandible. The mouth is chitinized along its upper edge, this brown, chitinous rim extending around the bases of the mandibles and bearing six toothlike lobes pointing downward along the portion of the edge between the mandibles (Pl. LII, fig. 1). A subdorsal and sublateral row of fine, white hairs runs the full length of the body on each side, one hair per segment in each row. The first three body segments bear several additional rows. What appears to be the anal segment is divided into a dorsal and a ventral lobe by a transverse invagination across the end. The dorsal lobe bears two pairs of short, fine hairs, one pair close together near each lateral end of the lobe. The ventral lobe bears a short hair at each lateral end. The body hairs are evidently tactile organs, since when any of them are touched, the larva wriggles and bites viciously at the point of contact.

Larvæ of this species seem to be better equipped, more vigorous, and more capable of defending themselves than the larvæ of *Micromelus subapterus* and *Merisus destructor*. *E. allynii* was reared from egg to adult on larvæ of both the other species just mentioned as well as on the Hessian fly. In one case, however, a newly hatched larva of *E. allynii* placed on a full-grown larva of *M. subapterus* in a glass cell was killed by the latter almost immediately. A few instances were observed where larvæ of *E. allynii* killed other individuals of the same species present in the same Hessian-fly puparium.

### THE PUPA

The larva forms a naked pupa (Pl. LI, fig. 3, 4) inside the puparium of the host. The first step in the process is the excretion of all waste matter from the body, leaving the larva pure white. The pupa is then formed and the last larval skin cast off. The newly formed pupa is nearly white, but turns dark within a few hours. The pupal stage of 30 specimens reared in glass cells varied from 9 to 24 days. The pupal period of those pupating in the summer averaged 13 days, while the pupal periods of those reared late in the fall became as long as 24 days in some cases. The arrival of cold weather retards pupal development, but whether or not the pupæ are able to survive severe winter temperatures has still to be determined. When the adult has completely developed, the pupal skin is cast off inside the host puparium, and the adult gnaws a round hole through the flaxseed near one end, penetrating the leaf sheath covering the flaxseed, through which it emerges.

### THE ADULT

After remaining quiet until dry, the adult becomes very active. Adults do not seem to fly more than a few feet at a time, using their wings merely to go from stem to stem. They do this so quickly and often that it is difficult to observe a single individual in the field very long. The females run quickly up and down the wheat stems, vibrating their antennæ rapidly against the side of the stem until they come to a place where a Hessian-fly puparium is located. Here they feel back and forth above the flaxseed until they locate the exact spot which suits them for oviposition. Then, facing upward, the tip of the abdomen is bent down until it touches the stem and raised away again, leaving the ovipositor pressed vertically against the stem supported from its articulation with the middle ventral portion of the abdomen. The leaf sheath and puparium are pierced by what under the microscope appears to be a sort of drilling motion of the ovipositor, which seems to be rotated part way around and back again. Oviposition takes several minutes.

Males placed in the same cage with females usually attempt to mate with them at once. Mr. W. R. McConnell has ascertained that this species can reproduce parthenogenetically. The question of the sex

of parthenogenetic progeny has not yet been definitely settled. Mated females produced both male and female progeny. Two mated adults kept separately in vial cages from the time they emerged from pupæ until they died each laid a total of 58 eggs. This number actually was found in each case by dissection of flaxseeds which had been exposed to the adult. A few eggs may have been lost in dissection. These adults remained alive for periods of 48 and 56 days and were ovipositing during periods of 29 and 46 days, respectively. Another adult, caught in the field while ovipositing in a flaxseed, remained alive in a vial cage and oviposited in flaxseeds during a period of 57 days. An unmated female was kept alive in a vial cage for 83 days. How long adults normally live in the field is not known.

In one experiment Mr. W. H. Larrimer, of the Bureau of Entomology, exposed stems of *Elymus canadensis* containing galls of *Isosoma* sp. to two *Eupelmus allynii* females which previously had been ovipositing in Hessian-fly puparia. They at once oviposited in the galls. The galls were dissected and the inclosed larvæ of *Isosoma* sp., together with the eggs of *E. allynii* found in the galls, were transferred to glass-cell cages, one larva of *Isosoma* sp. and one parasite egg to each cell. The parasites proceeded to complete their development to adults on the larvæ of *Isosoma* sp. Progeny were also bred on the Hessian fly from the same parents used by Mr. Larrimer. These parents and their progeny were all determined by Mr. Gahan as *E. allynii*.

## MERISUS DESTRUCTOR

### THE EGG

The egg of *Merisus destructor* Say (Pl. LI, fig. 5) is elongate, kidney-shaped, circular in cross section, with one end smaller than the other. It is white, with the surface apparently smooth. The average length of eggs measured was 0.4 mm., the average diameter at thickest point, 0.1 mm. Hundreds of the eggs were dissected from flaxseeds, in which they had been deposited, and in all cases they were found external to the host larva or pupa inside the puparium. Some eggs apparently bore a short pedicel on one end, which seemed to be fastened to the inside of the host puparium. Ordinarily, however, the eggs were found free.

*M. destructor*, like *E. allynii*, normally oviposits in the Hessian-fly flaxseed, according to the observations of Mr. Kelly and the author at Wellington, Kans. It was very abundant in the fields at times when no other stage of the Hessian fly was present. The females were repeatedly observed ovipositing in puparia in the field. In cages they also oviposited readily in flaxseeds contained in sections of wheat stems as well as in naked flaxseeds removed from stems. They did not oviposit readily in sections of stems containing only partially grown Hessian-fly larvæ, although they seemed interested in them. In one instance,

however, a female *M. destructor* oviposited in a stem containing nothing but partially grown larvæ. Upon dissection the egg was found sticking to the stem underneath the leaf sheath, close to one of the larvæ. It is not yet known whether or not *M. destructor* can develop to maturity on partially grown Hessian-fly larvæ. The egg stages of 96 specimens placed on Hessian-fly larvæ in glass slides varied from 1½ days in hot July weather to 4 days in cool September weather. The larva emerges from the egg by breaking through one end. After crawling around a little the larvæ reared in glass cells fastened themselves with their mandibles to the outside of the host larvæ in order to feed.

### THE LARVA

The full-grown larva of *M. destructor* (Pl. LII, fig. 4) is white with the dingy brown contents of the alimentary tract visible through the integuments. There are two pairs of slightly raised circular tubercles on the front of the head near the top. The lower pair are slightly farther apart than the upper pair and each bears a small conical projection, evidently an antenna, varying from white to pale brown in color and about 0.02 mm. long. The median ventral surface of the head bears the round suctorial mouth opening. The only mouth appendages distinguishable are a pair of brown chitinous mandibles borne laterally and closing together across the mouth with their tips overlapping (Pl. LII, fig. 3). The distal portion of the mandible is conical, tapering gradually to a sharp point. The proximal end is suddenly enlarged, evidently to provide for muscle fastenings. One subdorsal and one sublateral row of very short and inconspicuous setæ on each side of the body are clearly distinguishable in some specimens, extending the full length of the body, one seta per segment in each row. On some specimens there appear to be two ventral and two dorsal rows of scarcely discernible setæ on the first three body segments only. There are thirteen body segments besides the head, the anal segment being divided into a dorsal and a ventral lobe by a horizontal fold across the end. The dorsal lobe bears four very short, fine setæ in a row across the end, the setæ composing the row being usually in two lateral pairs. The ventral anal lobe bears only two setæ, one near each lateral end of the lobe. The length of the full-grown larvæ averages 2.5 mm., the largest diameter, 0.7 mm.

Balsam mounts of all the material left behind in the little glass cells by pupating larvæ nearly always contained five pairs of mandibles. Mounts of all the material left in the cell by full-grown larvæ which had ceased to feed, together with the mandibles dissected from such larvæ, also nearly always contained five pairs of mandibles. In every mount the pairs varied uniformly in size from those resembling the ones borne by newly hatched larvæ to those borne by full grown larvæ. Mandibles of newly hatched larvæ were somewhat hooked. All the remaining pairs were similar in shape, and corresponding pairs in all the mounts

were almost identical in size. As was the case with *E. allynii*, the mandibles of the newly hatched larva appeared to be heavier, more powerful, and somewhat larger than the mandibles borne by the second-instar larva. Also, the head shield appeared to be more heavily chitinized in the first instar than in the later ones. Beginning with the second instar, the successive pairs of mandibles apparently increase fairly uniformly in size with each molt. In the mounts where five pairs of mandibles could not be found, those which were found correspond in size and shape to some one of the pairs in the complete series and it was evident that certain pairs had been lost in making the mount. No more than five pairs were found in any one case. All the findings lead to the conclusion that larvæ of *M. destructor* normally pass through five instars in making their growth.

The relative sizes of the molted mandibles are shown below. The measurements represent the distance in a straight line from the tip of the mandible to the shoulder where it suddenly enlarges into the broad base.

| Molt No. | Length of mandible. |
|---|---|
| 1 | 0.014 mm. |
| 2 | .014 mm. |
| 3 | .020 mm. |
| 4 | .024 mm. |
| 5 | .032 mm. |

The larvæ develop readily on Hessian-fly larvæ and pupæ, both in flaxseeds and in glass cells, unless the host pupa has nearly completed its development. Several newly hatched larvæ in flaxseeds and glass cells containing Hessian-fly pupæ which were nearly developed killed the pupæ, but died from lack of sufficient food to complete their growth. The larvæ are evidently cannibalistic upon occasion. In one flaxseed which had been exposed to ovipositing females, a young larva of *M. destructor* was found which had been feeding, as also the shrunken remains of another young larva. Evidently the healthy larva had found and killed the other and was feeding on the Hessian-fly larva when the flaxseed was dissected. Full-grown larvæ in glass cells punctured and killed eggs and larvæ of *M. destructor* which were placed in the cells with them. Larvæ of *M. destructor* were able to become full grown by feeding on larvæ of *M. subapterus* also.

The periods required by 36 larvæ to make their growth when reared in glass cells varied from 7 to 11 days. Cool weather appeared to make growth slower. After becoming full-grown the majority of the larvæ of *M. destructor* reared in glass cells remained quiescent for months, though still alive and able to wriggle vigorously when touched. Larvæ reared in flaxseeds exhibited the same characteristic. In other words, the larvæ seem to have a tendency to estivate and hibernate until another warm season before pupating. Larvæ of *M. destructor* were actually found to

have hibernated in stubble of wheat cut the previous June. Eight per cent of the flaxseeds in stubble gathered from a field in southeastern Kansas in late March contained live, full-grown parasite larvæ which afterwards became adult and were determined by Mr. Gahan as *M. destructor*.

### THE PUPA

The period from the formation of the pupa (Pl. LI, fig. 6) to the emergence of the adult varied from 7 to 14 days in 21 specimens carried through this stage in glass cells. Those pupating in April and September, when cooler temperatures prevailed, took longer to develop than those which pupated during the hot weather of July and August. The larvæ form naked pupæ inside the puparium of the host. The process of pupation as observed in glass cells begins with the excretion of all waste matter from the body of the larva, which then becomes pure white. In a few hours the pupa is formed. The eyes begin to turn reddish in about a day and by the fourth day are a very dark red. The body of the pupa is by the fourth day a creamy white, and by the sixth day the head and thorax are black. Within another day the abdomen turns black except for the base of the abdomen, which assumes the light brown as found in males and some females. The emergence of the adult follows within a day or so after the pupa has turned dark. Cool weather retards development. The adult casts off the pupal skin inside the host puparium and emerges by gnawing a round hole through the side of the flaxseed and the wheat leaf sheath covering it just large enough for the adult parasite to crawl through.

### THE ADULT

Adults soon become active after emerging from flaxseeds. In the spring males emerged two or three days before the females in cages containing stubble collected from the fields where it had stood during the winter. Mating took place at once when the females emerged. Oviposition takes place in the following manner: The females run up and down the wheat stalks, vibrating their antennæ rapidly against the side of the stem. When they come to a place where there is a flaxseed underneath the leaf sheath, they stop and excitedly feel up and down over the place where the flaxseed is located. They face upward to oviposit, with the body parallel to the puparium. They locate the proper place for oviposition with the tip of the abdomen and then raise it away from the stem, leaving the ovipositor unsheathed and pointing perpendicularly against the stem from its articulation with the middle of the abdomen. In less than a minute the ovipositor is forced through the leaf sheath and the puparium. In penetrating the flaxseed the ovipositor is seemingly rotated like a drill part way round and back again. Oviposition takes 5 to 10 minutes, and dissections of flaxseeds indicate that a single egg is laid at a time. One female kept isolated in a vial cage laid a total of 39 eggs in puparia exposed

to her and later dissected. Some may have been lost in dissection. This female was laying eggs during a period of six weeks. Other females were kept alive and active in confinement for periods of over two months.

Some stems of *Elymus canadensis* containing galls formed by a species of Isosoma were placed in a vial cage containing females of *M. destructor*. Almost immediately one of the females became interested in the galls, feeling over them with her antennæ. She then attempted to oviposit, endeavoring persistently to penetrate the gall with her ovipositor, but without success. Mr. W. H. Larrimer finally succeeded in getting the females to oviposit in the Isosoma galls and found the eggs inside the galls but external to the larvæ of *Isosoma* sp. He actually reared a few specimens of *M. destructor* from egg to adult on the Isosoma larvæ in glass cells. The parents used in this experiment and the progeny which were reared were determined as *Merisus destructor* by Mr. Gahan.

## MICROMELUS SUBAPTERUS[1]

Heretofore it has been uncertain that the winged and wingless forms of *Micromelus subapterus* Riley were the same species. It has been proved, however, that the two forms are specifically identical by breeding a wingless female from a winged parent. Further evidence indicating that the winged and wingless forms are the same species is the fact that wingless males mated with winged females as readily as with the wingless form. The method by which the wingless female was bred from the winged parent is as follows: The winged parent deposited an egg in a Hessian-fly puparium known to have been previously unparasitized. The egg was removed from the puparium and from it a wingless adult was reared on a healthy Hessian-fly larva, which also had been dissected from its puparium. Mr. Gahan found this wingless offspring of a winged adult to be identical with winged specimens of unknown parentage.

### THE EGG

The egg of *Micromelus subapterus* (Pl. LI, fig. 7) resembles that of *Merisus destructor* in size and shape. It is elongate, kidney-shaped, with one end longer than the other, circular in cross section, white in color, with surface of shell smooth, and about 0.38 mm. long by 0.09 mm. in diameter at the thickest point. It has no stalk.

All the observations made at the Wellington (Kans.) station lead to the conclusion that the egg is normally laid in the Hessian-fly puparium. In cages the adults oviposit readily in flaxseeds, the eggs being placed inside the puparia but external to the inclosed Hessian-fly larvæ and unattached. This was the case both when stems of fly-infested wheat

[1] Mr. A. B. Gahan makes the following statement: "The real generic position of this species is in doubt. It was originally described by Riley under the name *Merisus* (*Homoporus*) *subapterus* Riley, and later referred to Boeotomus by Osborn and other writers. N. V. Kurdiumov has more recently placed the species in the genus Micromelus. Doctor Ashmead reduced Boeotomus to synonymy with *Micromelus*."

were exposed to the parasite in vial cages and when ovipositing females were placed in large glass chimneys containing growing wheat infested with the Hessian fly. Occasionally the females have been observed apparently to oviposit in stems containing only larvæ; and although careful dissections of these stems were made, no eggs were found. Further proof that *M. subapterus* normally oviposits in flaxseeds was obtained by dissecting puparia collected in fields where this parasite was numerous at the time the collection was made. Both eggs and young larvæ of a parasite were present in the flaxseeds and when reared to maturity in the laboratory were found to be *M. subapterus*. The egg stage in 119 cases varied from 1½ to 5 days. Low temperatures in fall and spring retarded embryonic development. The larvæ reared in glass cells emerged from the eggshells by breaking through one end, and after crawling around a short time settled down in one place to feed.

### THE LARVA

The full-grown larva of *M. subapterus* (Pl. II, fig. 6) averages 2 mm. long by 0.75 mm. in thickness. It is white, with the pale-brown contents of the alimentary tract showing through the body. There are two pairs of slightly raised circular tubercles on the front of the head near the top. The lower pair are slightly farther apart than the upper pair, the former each bearing a small conical projection, evidently the antennæ, varying from white to brown and about 0.015 mm. long. The median ventral surface of the head bears the round, suctorial mouth opening. The only mouth appendages distinguishable are a pair of very small brown chitinous mandibles borne laterally and closing together across the mouth (Pl. LII, fig. 5). The distal ends of the mandibles are sharp and needlelike. The proximal ends are suddenly enlarged, evidently to provide for muscle fastenings. A minute pit, which sometimes appears to have a brown center, occurs on each side of the mouth. The body is entirely glabrous, so far as could be determined, except for the anal segment, oval in shape, with the anal end the more pointed. There are 13 segments besides the head, the anal segment being divided into a dorsal and ventral lobe by a horizontal fold across the end. The dorsal lobe bears four short, very fine setæ in a transverse row, these usually being in lateral pairs. The ventral anal lobe bears only two very short, fine setæ, one near each lateral end of the lobe.

The number of instars passed through by larvæ of *M. subapterus* in making their growth appeared to be five. Five pairs of molted mandibles increasing uniformly in size, from the small pair resembling those borne by newly hatched larvæ to the large pair molted off when full-grown larvæ pupated, were present in almost every mount made of the material left behind in a cell where a larva had developed. In

mounts where five pairs could not be found, each of those present corresponded to some one of the pairs in the complete series. No more than five pairs were found in a single mount. As in the two species of parasites previously discussed, the head shield of the newly hatched larva was more heavily chitinized than those of later instars. The mandibles appeared to be more powerful for their size than those of any later instar, and in some cases they were actually larger than the second-instar mandibles. The approximate sizes of the respective pairs of molted mandibles follow. The measurements represent the distance from the tip of the mandible to the shoulder where it suddenly enlarges into the broad base.

| Molt No. | Length of mandible. |
|---|---|
| 1 | 0.012 mm. |
| 2 | .012 mm. |
| 3 | .016 mm. |
| 4 | .020 mm. |
| 5 | .028 mm. |

Larvæ of *Micromelus subapterus* do not seem as capable of moving around and reattaching themselves to the host as are the larvæ of *Eupelmus allynii* and *Merisus destructor*. Larvæ reared in glass cells crawled about a little immediately after hatching before they settled down to feed, but they usually completed a large part of their growth without leaving the original feeding point on the external surface of the host.

This species not only developed on Hessian-fly larvæ in puparia, but in some instances fed on the larvæ of other parasites. One egg of *Micromelus subapterus* was placed on a full-grown larva of the same species in a glass cell. The egg hatched and the little larva became full grown on the large larva, almost completely devouring it. Another egg of *M. subapterus* was placed on a full-grown larva of *Merisus destructor* and the little larva hatching from the egg became full grown on the larva of *Merisus destructor*. Experiments like these, however, usually resulted in the destruction of the egg or young larva of *M. subapterus* and the survival of the full-grown larva of the same or the other species as the case happened to be. Larvæ of *M. subapterus* apparently could make their growth on the Hessian-fly pupa as well as on the larva unless the former had partially developed. Where the host pupa had already completed a large part of its development, both the host and the parasite generally died, the latter apparently for lack of sufficient suitable food. Larvæ of *M. subapterus* appeared to be the least able to defend themselves where the larvæ of more than one species occurred in the same flaxseed. They also seemed the least capable of successfully establishing a feeding point on the host larva, at least when reared in little glass cells. They seemed more delicate in structure and less vigorous.

The respective periods required for 36 larvæ to make their growth varied from 7 to 10 days. A large proportion of the larvæ after finishing

their growth remained in a quiescent state in the little glass cells for months. Others pupated at once upon completing their growth.

### THE PUPA

In general, the process of pupation as observed in glass cells is as follows: The full-grown larva excretes all waste matter from the body, leaving it perfectly white. Within a day after this operation the pupa (Pl. LI, fig. 8) is formed and is at first perfectly white, the last larval skin being found at the anal end of the pupa. In another day or so the pupa begins to turn a pale brown, and the eyes turn reddish. The pupa finally becomes entirely black as development progresses, the head and thorax changing first, and remains so until the adult emerges.

The pupa is formed naked inside the puparium of the host. The adult emerges by casting off the pupal skin inside the host puparium and then cutting a round hole through the side of the flaxseed near one end. The length of the pupal period varied in 21 instances from 7 to 13 days. Cool weather retarded the development of the pupæ. A larger proportion of the larvæ reared in the cooler weather of fall pupated at once upon attaining their growth than was the case with the larvæ reared in the hot weather of midsummer, indicating a tendency of the larvæ of this species to estivate.

### THE ADULT

Newly emerged adults became active almost at once upon emerging from the host puparium. Males placed in the same cage with females began mating at once. Females that had been mated seemed to oviposit more readily than unmated females. Both Mr. McConnell and the writer found that this species was arrhenotokous in every instance where this point was determined. Females have been kept alive in cages as long as six months, and one female oviposited after having been kept alive over five months. It was usual for them to live and oviposit for at least a month in vial cages. One female actively oviposited during a period of 75 days and laid a total of 103 eggs. Another female laid a total of 45 eggs. The number of eggs laid by a single female was determined by exposing flaxseeds to an isolated individual and dissecting them to find the number of eggs the parasite had laid in each.

In ovipositing the female would run up and down the stems of the plants, vibrating her antennæ against the surface. When she came to a place in the stem where a flaxseed was located, she would stop, feel up and down over the spot with her antennæ, and then lower the tip of her abdomen. When she had found the point that suited her for oviposition, the end of the abdomen was raised, leaving the ovipositor standing vertically against the side of the stem from its articulation with the middle of the abdomen. In penetrating the leaf sheath and puparium the parasite seemed to rotate the ovipositor with a drilling motion in

addition to the downward pressure exerted on it. The female always took a position heading up the stem in ovipositing. The whole process generally took five minutes or more.

## CONCLUSION

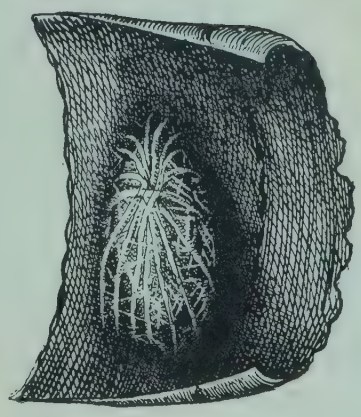
The writer's experiments and observations have all led to the inference that only one specimen of any of the three species studied ever matures in a single Hessian-fly puparium. In every instance where more than one egg or larva was placed on the same host or in the same cell, one survived and the rest were killed by that one, or starved to death. This was true whether the two or more larvæ were of the same or different species.[1]

[1] For correct figures of the adults of all three of the species treated in this paper, see U. S. Dept. Agr. Farmers' Bul. 640. (Webster, F. M. The Hessian fly. 20 p., 17 fig. 1915.)

## PLATE LI

Fig. 1.—Egg of *Eupelmus allynii*.
Fig. 2.—Egg of *Eupelmus allynii* in situ.
Fig. 3, 4.—Pupa of *Eupelmus allynii*.
Fig. 5.—Egg of *Merisus destructor*.
Fig. 6.—Pupa of *Merisus destructor*.
Fig. 7.—Egg of *Micromelus subapterus*.
Fig. 8.—Pupa of *Micromelus subapterus*.

8



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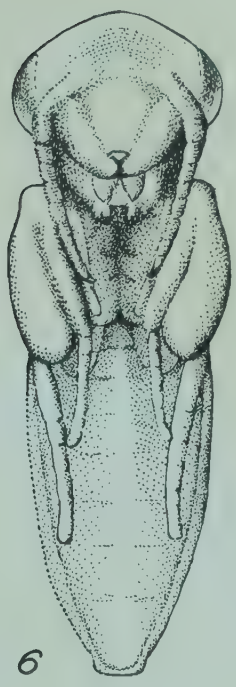

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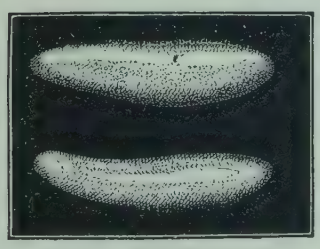

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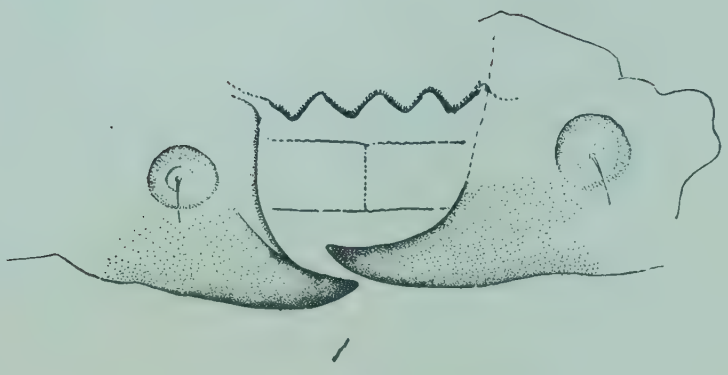
PLATE LII



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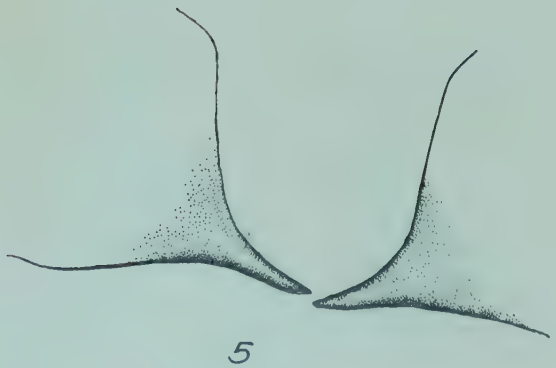

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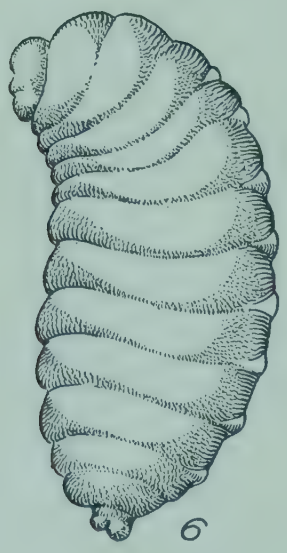

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## PLATE LII

Fig. 1.—Mandibles of full-grown larva of *Eupelmus allynii*.
Fig. 2.—Larva of *Eupelmus allynii*.
Fig. 3.—Mandibles of full-grown larva of *Merisus destructor*.
Fig. 4.—Larva of *Merisus destructor*.
Fig. 5.—Mandibles of full-grown larva of *Micromelus subapterus*.
Fig. 6.—Larva of *Micromelus subapterus*.

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# EFFECT OF RÖNTGEN RAYS ON THE TOBACCO, OR CIGARETTE, BEETLE AND THE RESULTS OF EXPERIMENTS WITH A NEW FORM OF RÖNTGEN TUBE

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## INTRODUCTION

The Röntgen tube used in experiments on the effect of Röntgen rays on the tobacco, or cigarette, beetle (*Lasioderma serricorne* Fabricius) described in this paper is a new form designed by Coolidge.[1] By this type of tube a much more powerful Röntgen-ray radiation can be maintained than was possible with the apparatus used in experiments of a similar nature previously made by the writer. The intensity and the penetrating power of the Röntgen rays produced are both under the complete control of the operator, and many of the factors limiting the use of other types of tubes for the special purpose desired are absent. The tube can be operated continuously for long periods without showing an appreciable change in either the intensity or the penetrating power of its resulting radiation. The starting and running voltage are the same. The resulting radiation is therefore homogeneous and of any desired penetrating power.

The ordinary forms of tubes used in previous experiments were incapable of being operated continuously without change in penetrating power. Owing to the fluctuation in intensity and penetrating power incidental to frequent adjustment, it was impossible to tell with any degree of accuracy the dosage and amount of radiation.

In previous experimental work with Röntgen rays it had been found that in sterilizing cigars or tobacco, small dosages are ineffective, from a practical standpoint. To be effective, the radiation must be intense, and it is evident that if the process can be successfully applied to commercial work, the apparatus used must be capable of producing and maintaining such radiation during the entire period required for the material treated to pass through the exposure chamber of the machine.

[1] Coolidge, W. D. A powerful Röntgen ray tube with a pure electron discharge. *In* Phys. Rev., s. 2. v. 2, no. 6, p. 409-430, 6 fig. 1913.

TABLE I.—*Effect of Röntgen rays on development of the tobacco beetle*

[Experiments made at Schenectady, N. Y.[1]]

| Experiment No. | Date. | Stage of insect. | Number tested. | Age. | Current (milliampere minutes). | Time. | Exposure (milliampere minutes). | Date examined. | Results. | Date examined. | Results. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Days.* | | *Minutes.* | | | | | |
| 1 | Apr. 10 | Egg | 27 | 1 | 10 | 15 | 150 | Apr. 16 | None hatched | May 1 | None hatched. |
| 2 | Apr. 12 | do | 95 | 1–2 | 15 | 10 | 150 | Apr. 20 | do | do | Do. |
| 3 | Apr. 13 | do | 35 | 1 | 15 | 10 | 150 | May 1 | do | Sept. 11 | Tobacco uninjured and uninfested. Check heavily infested. |
| 4 | Apr. 14 | do | 50 | 1 | 15 | 10 | 150 | do | do | do | Do. |
| 5 | Apr. 15 | do | 50 | 1 | 15 | 10 | 150 | do | do | do | Do. |
| 6 | do | do | 50 | 3 | 15 | 10 | 150 | do | do | do | Do. |
| 7 | Apr. 16 | do | 50 | 2 | 15 | 10 | 150 | do | do | do | Do. |
| 8 | do | do | 110 | 1 | 15 | 10 | 150 | do | do | do | Do. |
| 9 | do | do | 50 | 2 | 15 | 10 | 150 | do | do | do | Do. |
| 10 | do | do | 50 | 2 | 15 | 10 | 150 | do | do | do | Do. |
| 11 | Apr. 17 | do | 50 | 3 | 15 | 10 | 150 | do | 1 eggshell and 1 live larva | do | 1 dead larva partly grown. Check infested. Larvæ, pupæ, and adults found. |
| 12 | do | do | 50 | 3 | 15 | 10 | 150 | do | None hatched | do | No signs of development. Tobacco uninjured. Check infested. |
| 13 | do | do | 42 | 4 | 15 | 40 | 600 | do | do | do | Do. |
| 14 | do | do | 52 | 5 | 15 | 40 | 600 | do | 38 eggs hatched; 14 sterile | do | Several small dead larvæ found; no pupæ or adults; no evidence of any of the larvæ having developed. |
| 15 | do | do | 52 | 1 | 15 | 10 | 150 | do | None hatched | do | Tobacco uninfested and uninjured. Check box infested. |
| 16 | June 7 | do | 203 | 1–2 | 15 | 10 | 150 | June 24 | do | Sept. 1 | Do. |
| | | | | | | | | July 11 | do | Dec. 12 | No evidence of any of the eggs having hatched. |
| 17 | do | do | 20 | 1–3 | 15 | 10 | 150 | June 24 | do | Sept. 1 | Do. |
| | | | | | | | | July 11 | do | Dec. 12 | Do. |
| 18 | do | do | 82 | 4–5 | 15 | 10 | 150 | June 24 | 2 dead larvæ in first instar; a few eggshells; no live larvæ. | Sept. 1<br>Dec. 12 | No signs of further development. Tobacco uninfested. |
| 19 | Apr. 17 | Larval | 21 | (2) | 15 | 40 | 600 | May 3 | All alive | | |
| | | | | | | | | May 28 | 14 alive; 7 dead | | |
| | | | | | | | | July 11 | 2 alive; rest dead | | |
| | | | | | | | | Sept. 1 | All dead; none reached adult stage; all check larvæ transformed by July 11. | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | June 7 | ...do..... | 35 | (3) | 15 | 40 | 600 | June 24 | 21 alive; part seem inactive..... | Sept. 1<br>Oct. 1<br>Nov. 1<br>Dec. 11<br>Jan. 1, 1916.<br>Jan. 10, 1916. | 9 alive...<br>5 alive...<br>4 alive...<br>2 alive...<br>2 alive...<br>All dead | No signs of feeding observed.<br>None of the larvæ reached the pupal stage. Treatment apparently stopped all development and activity; were somewhat shrunken and of an abnormal whitish color. |
| | | | | | | | | July 11 | 12 alive, but in a dormant condition; none have transformed to pupal or adult stage; checks have all transformed to adults. | | | |
| 21 | ...do..... | Pupal... | 20 | ..... | 15 | 40 | 600 | June 16<br>July 11 | 8 alive; none transformed.....<br>4 live adults found..... | Aug. 1 | Adults dead. No eggs laid. | |
| 22 | Apr. 17 | Adult... | 67 | ..... | 15 | 40 | 600 | Apr. 17 | Alive and active directly after exposure. | Oct. 1 | Tobacco from jar containing exposed beetles and eggs examined; no signs of infestation. | |
| | | | | | | | | Apr. 22 | Large number of eggs deposited. 208 eggs kept under daily observation; rest of eggs placed with chewing tobacco in sealed jar. | | | |
| | | | | | | | | May 30 | None of the eggs hatched; eggs from check beetles hatched normally. | | | |
| 23 | June 7 | ...do..... | 50 | ..... | 15 | 40 | 600 | June 24 | 26 alive, 24 dead; large number of eggs found; none hatched; check beetles, 32 alive, 18 dead; eggs from check beetles hatched normally. | July 24 | All adults in both exposed and check lot dead; none of the eggs from exposed beetles hatched. | |
| | | | | | | | | July 11 | Exposed lot, 7 alive; check, 5 alive; 126 eggs kept from exposed lot examined; none hatched. | Sept. 1 | No signs of infestation in tobacco kept in jar which contained exposed adults. Tobacco in check jar infested. | |

[1] The following were constant in all the experiments: Material exposed 7.5 inches from focal spot of tube; spark gap 5.5 inches, giving, at a humidity of 57°, a voltage of about 65,000.

[2] Partly grown.

[3] Partly grown; some nearly mature.

## EXPERIMENTAL WORK

Eggs for the experiments were obtained by placing large numbers of tobacco beetles in jars containing leaf tobacco which had been sterilized by heat. The eggs were then placed between slabs of chewing tobacco in wooden boxes. The covers of the boxes were tightly sealed with adhesive tape. Control boxes containing approximately the same number of eggs as the treated boxes were prepared in a similar manner.

Infested tobacco containing larvæ, pupæ, and adults was also exposed in sealed wooden boxes. After exposure the insects were transferred to wooden boxes containing granulated tobacco which had been sterilized by heat. A corresponding number of specimens were kept as controls.

Exposure to the rays was made by placing the containers directly under the Röntgen tube at a distance of 7.5 inches from its focal spot. In order to guard against any effect of heat, a fan was kept blowing on the container while the exposure was made. The maximum temperature registered by a thermometer placed in the chamber was 91° F.

In the series of experiments tabulated 150 milliampere minutes (current of 15 milliamperes for 10 minutes or a current of 10 milliamperes for 15 minutes), with a voltage of 65,000, was the minimum dosage applied.

The material used in the experiments was kept under observation until January 10, 1916. Table I gives the details of the experiments. The notes included show the condition at different times. During the colder months the material was kept in an automatically regulated electric incubator in which suitable breeding conditions were maintained. The temperature was kept at 86° F. and the humidity at 80.

Eggs from exposed beetles were kept under daily observation. Part were kept in cells on microscope slides and part were kept on the leaf tobacco on which they were laid and placed between slabs of chewing tobacco. Most of the eggs which failed to hatch became shrunken and changed in color in about 10 days. Part remained plump and apparently normal for a considerable time. In eggs which were over 2 days old and in which embryonic development was well advanced when treated the partly developed larvæ could be seen within by examination with a microscope.

As will be seen in Table I (experiments 11, 14, and 18), hatching took place in some of the eggs which were over 3 days old. In experiment 14, which was made with eggs nearly hatched when treated, part of the eggs hatched, even though the dosage of 150 milliampere minutes, which was effective with the newly laid eggs, had been increased to 600 milliampere minutes.

Results of previous experiments, as well as those tabulated, indicate that in treatment of the egg stage heavier dosages are required to sterilize eggs which are nearing the end of the incubation period than are required to sterilize eggs newly laid.

In these experiments the larvæ hatched from treated eggs failed to develop. In several other series of experiments with Röntgen rays made by the writer and also in experiments made by Morgan and the writer,[1] eggs given lighter dosage hatched and development seemed normal, several generations of tobacco beetles being reared from some of the tobacco and cigars which contained treated eggs.

In the two experiments with larvæ (No. 19 and 20), no immediate effect as the result of exposure to the rays was noted. After a time the larvæ became inactive, somewhat shrunken, and changed in color, and no evidence of feeding could be observed. Nearly all remained in an inactive or dormant condition for long periods before death. Two larvæ exposed on June 7 (experiment 20) remained alive until January 10, 1916. All check larvæ used in this experiment had transformed to the adult stage by July 11. All treated larvæ died before reaching the pupal stage. With conditions under which the material used in the experiments was kept, the normal larval period of the tobacco beetle is about 40 days. All larvæ used in the experiments were partly grown when the experiment was made. No further growth could be noticed. In general, the effect of the heavy exposure given (600 milliampere minutes, voltage 65,000, distance from focal spot of Röntgen tube 7.5 inches) seems to have been to stop development and activity and to produce an inactive or dormant condition, and greatly to prolong the larval period.

The results of all previous experiments with larvæ given comparatively light exposures had shown entirely negative results.

In the experiment with pupæ (experiment 21) the number of pupæ used was not sufficiently large to permit the drawing of positive conclusions. Of the 20 specimens treated, only 4 reached the adult stage. These seemed normal, but died without laying eggs.

In the two experiments with adults (experiments 22 and 23), the results obtained were very similar. The exposure given apparently did not affect the length of life or the activity. Mating was observed and large numbers of eggs were laid. None of the eggs from the exposed beetles hatched, while eggs from the check beetles hatched normally.

Egg clusters of the tent caterpillar (*Malacosoma americana* Fabricius) and the white-marked tussock moth (*Notolophus leucostigma* Smith and Abbot) were used. With both of these species the period of incubation is very long, eggs deposited in summer or fall not hatching until the following season. An exposure of 150 milliampere minutes was given. Other conditions of the experiment were the same as in experiment 7 made with eggs of the tobacco beetle, details of which are given in Table I. The experiment was made on April 16. The egg clusters treated contained something over 1,000 eggs of each species. The same number of clusters were kept as checks. Both experiments gave nega-

[1] Morgan, A. C., and Runner, G. A. Some experiments with Röntgen rays upon the cigarette beetle Lasioderma serricorne Fabr. *In* Jour. Econ. Ent., v. 6, no. 2, p. 226–230. 1913.

tive results, hatching being apparently normal in treated eggs of both species.

The eggs of both the tent caterpillar and the tussock moth were nearing the end of the incubation period when treated. In eggs of the tent caterpillar embryonic development is practically completed in the fall, the larvæ remaining in the eggshells over the winter and emerging on the appearance of warm weather in the spring.

## SUMMARY

Under laboratory conditions tests made with a Röntgen-ray tube permitting a high-energy input and giving an intense and powerful radiation gave results which promise that the X-ray process may be successfully used in treatment of cigars or tobacco infested with the tobacco, or cigarette, beetle.

Heavy dosages must be given, as is indicated by the exposure given in the series of experiments tabulated in this paper.

In treatment of the egg stage, heavier exposures are required to sterilize eggs which are near the hatching point than are required to sterilize eggs newly laid.

In experiments performed by the writer a dosage equivalent to 150 milliampere minutes exposure with a spark gap of 5.5 inches gave satisfactory results with eggs in tobacco placed 7.5 inches from the focal spot of the tube. With this exposure the eggs in which embryonic development was well advanced hatched, but in all cases where these larvæ were kept under observation they failed to reach the adult stage.

The minimum lethal dosage at a given distance from the focal spot of the Röntgen tube used has not been determined.

In two separate experiments adults were given an exposure of 600 milliampere minutes (amperage × time), with a spark gap of 5.5 inches, giving an approximate voltage of 65,000, with humidity at 57. The distance from the focal spot of the Röntgen tube was 7.5 inches. The results are as follows:

(1) No effect on length of life was apparent, as the beetles died at about the same rate as the same number of beetles kept as a check.

(2) Large numbers of eggs were deposited after exposure. These eggs were infertile. Eggs laid by the check beetles hatched normally.

Larvæ were given an exposure of 600 milliampere minutes, other conditions of the experiment being the same as in the experiments with adults given above. While no immediate effect was apparent, the treatment had the effect of stopping activity and development, the larvæ remaining in a dormant condition for a prolonged period. All treated larvæ died before reaching the pupal stage.

# STIMULATING INFLUENCE OF ARSENIC UPON THE NITROGEN-FIXING ORGANISMS OF THE SOIL

By J. E. Greaves,
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## INTRODUCTION

Arsenic, when applied to a soil, has been found to stimulate the ammonifying (Greaves, 1913c)[1] and especially the nitrifying organisms of that soil. The stimulation varied greatly with the form, quantity, and method of applying the arsenic. Furthermore it was found that very large quantities of arsenic had to be applied to a soil before its toxic effect became marked. This toxic effect became pronounced only when quantities of arsenic which far exceeded those found in any of the cultivated soils (Greaves, 1913b) had been applied. Therefore it was desirable to determine its influence and mode of action upon the nitrogen-fixing powers of the soil. For, even though arsenic does not inhibit the action of the ammonifiers or nitrifiers, if it stops or materially retards the nitrogen-fixing organism, it can not be said that arsenic is not injurious to the soil flora. To determine this point the following study has been made.

## EXPERIMENTAL WORK

The soil used in the first part of this work was the same as that used by the author in the previous series. It is a typical bench soil, a sandy loam fairly high in calcium and iron content and supplied with an abundance of all the essential elements of plant food with the exception of nitrogen, which was low, a characteristic of arid soils.

The determination of the nitrogen-fixing powers of the soil was made as follows: Tumblers covered with Petri dishes were sterilized, and into these were weighed 100-gm. portions of the air-dried soil and 2 gm. of mannite, which were then carefully mixed. Sodium arsenate was added from a standard solution with the proper proportion of sterile distilled water and the mixture thoroughly stirred with a sterile spatula. The other arsenical compounds were added in the dry state and then carefully mixed. Sufficient sterile distilled water was added to make the moisture content of the soil 18 per cent. The tumblers and contents were weighed and the moisture content made up weekly to the initial concentration.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 414-416.

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The samples were incubated at 28° to 30° C. for 18 days and the total nitrogen determined. The tumblers and contents at the end of this time were placed in an electric incubator and kept at 95° C. until dry. The soil was then ground in a mortar, after which 20-gm. portions were weighed and placed in Kjeldahl flasks. The nitrogen was then determined according to the Lipman and Sharp (1912) method. The determinations were all made in duplicate and compared with sterile blanks, so that each result reported is the average of two or more closely agreeing determinations. The compounds used were sodium arsenate, lead arsenate, cupric aceto-arsenite (Paris green), arsenic trisulphid, and zinc arsenite. In each case the quantity of the compound added was such as to give equivalent quantities of arsenic. The results reported as milligrams of nitrogen per 100 gm. of soil are given in Table I.

TABLE I.—*Quantity of nitrogen (milligrams) fixed in 100 gm. of soil during 18 days with varying amounts and different forms of arsenic*

| Arsenic. | Sodium arsenate. | Lead arsenate. | Paris green. | Arsenic trisulphid. | Zinc arsenite. |
|---|---|---|---|---|---|
| *P. p. m.* | | | | | |
| 0 | 18.2 | 16.1 | 15.22 | 9.8 | 9.1 |
| 20 | 22.4 | 16.0 | 13.72 | 11.2 | 11.9 |
| 40 | 14.0 | 16.4 | 13.02 | 14.0 | 9.7 |
| 80 | 14.0 | 18.9 | 14.00 | 15.4 | 9.6 |
| 120 | 15.0 | 21.0 | 8.82 | 16.2 | 10.5 |
| 160 | 14.4 | 21.0 | 8.32 | 16.4 | 9.7 |
| 200 | 14.0 | 21.7 | 7.42 | 14.0 | 8.4 |
| 240 | 12.6 | 16.8 | 6.72 | 12.8 | 8.4 |
| 280 | 0 | 16.1 | 6.02 | 11.2 | 8.4 |
| 320 | 0 | 16.0 | 6.00 | 11.2 | 9.0 |
| 360 | 0 | 16.6 | 6.02 | 9.8 | 9.1 |
| 400 | 0 | 16.8 | 5.22 | 9.8 | 9.1 |
| 0 | 18.2 | 16.1 | 15.22 | 9.8 | 9.1 |

In this series the concentration of the arsenic was not carried above 400 p. p. m., for previous work had shown that the main stimulation occurs below this concentration. Furthermore the arsenic occurring in agricultural soils seldom exceeds 150 p. p. m., so it is likely that in agricultural soils it will never be found to exceed the quantity used in this work.

The results reported in the above table bring out some very interesting facts and show that the nitrogen-fixing organisms are very similar to the nitrifying organisms in so far as their relations to arsenic are concerned. The addition of 20 p. p. m. of sodium arsenate stimulates their action and 40 p. p. m. or more have a toxic influence. When the concentration of arsenic reaches 280 p. p. m., it stops all nitrogen-fixing activity. The toxic influence which becomes so very prominent above this concentration must be due entirely to the arsenic and not to the sodium ion, as Lipman and Sharp (1912) have added many times this

quantity of sodium in the form of sulphates, chlorids, and carbonates to the soil without retarding its nitrogen-fixing power.

The lead arsenate at the lower concentrations has no influence upon the nitrogen-fixing powers of the soil, but when the concentration reaches 80 p. p. m. a stimulating influence becomes quite perceptible. This continues until the concentration exceeds 200 p. p. m. Above this concentration the nitrogen fixed, within experimental error, is the same as that fixed in the untreated soil. It is interesting to note that the compound does not become toxic, even when the quantity added reaches 400 parts of arsenic per million parts of soil. This series shows a very close similarity to the nitrification series previously reported, and it is quite likely that part of the stimulating influence is due to the lead ion.

Paris green is toxic even in the lowest concentration used, and the toxicity increases as the quantity of Paris green added increases. This toxicity is due mainly to the copper ion. However, as was shown in the ammonification and nitrification work, the quantity of soluble arsenic present would be much higher where the Paris green was added than where the other compounds were used. The fact that no stimulation occurs in the Paris-green series points to the conclusion that the toxicity of the copper must increase much more rapidly than the stimulating influence of the arsenic. Yet it is quite possible that if a lower concentration of the substance had been taken a stimulation would have been noted.

Arsenic trisulphid stimulates in the lowest concentration tested and increases in stimulating influence until a concentration of 160 p. p. m. is reached. In concentrations above this its stimulating influence decreases. In concentration above 320 p. p. m. there is fixed no more nitrogen in the presence than in the absence of arsenic. But even at the highest concentration tested (400 p. p. m.) this compound exerts no tonic influence on the nitrogen fixers.

Zinc arsenite probably stimulates slightly in low concentrations, but aside from this it has little apparent influence on the nitrogen-gathering organisms. Had fresh soil been used in this series, greater stimulation would have been noted, as was found by later work.

The amount of nitrogen fixed in the untreated soil of the above series shows a marked variation. This is probably due to various factors, chief among which is the fact that the nitrogen-fixing powers of the soil with sodium arsenate, lead arsenate, and Paris green were made in the order named on the air-dried soil soon after it had been brought to the laboratory. In the case of the arsenic trisulphid and zinc arsenite the soil had been in the laboratory in an air-dried condition for about two months before the determinations were made, but each set of samples within each series was handled in exactly the same manner, and the samples are directly comparable within each set, as has been the case in the previous

discussion. In order to make those containing different forms of arsenic more nearly comparable with each other—that is, the lead arsenate with the arsenic trisulphid, etc.—the nitrogen fixed in the untreated soil has been taken as 100, and from this the ratio has been calculated with each of the concentrations of arsenic. This gives us more nearly comparable results, which are shown in figure 1.

Comparing these results with those obtained for the ammonification and nitrification series (Greaves, 1913c), we find a marked similarity existing between them. In all of the series there is a marked stimulation with all of the compounds except Paris green. The arsenic trisulphid

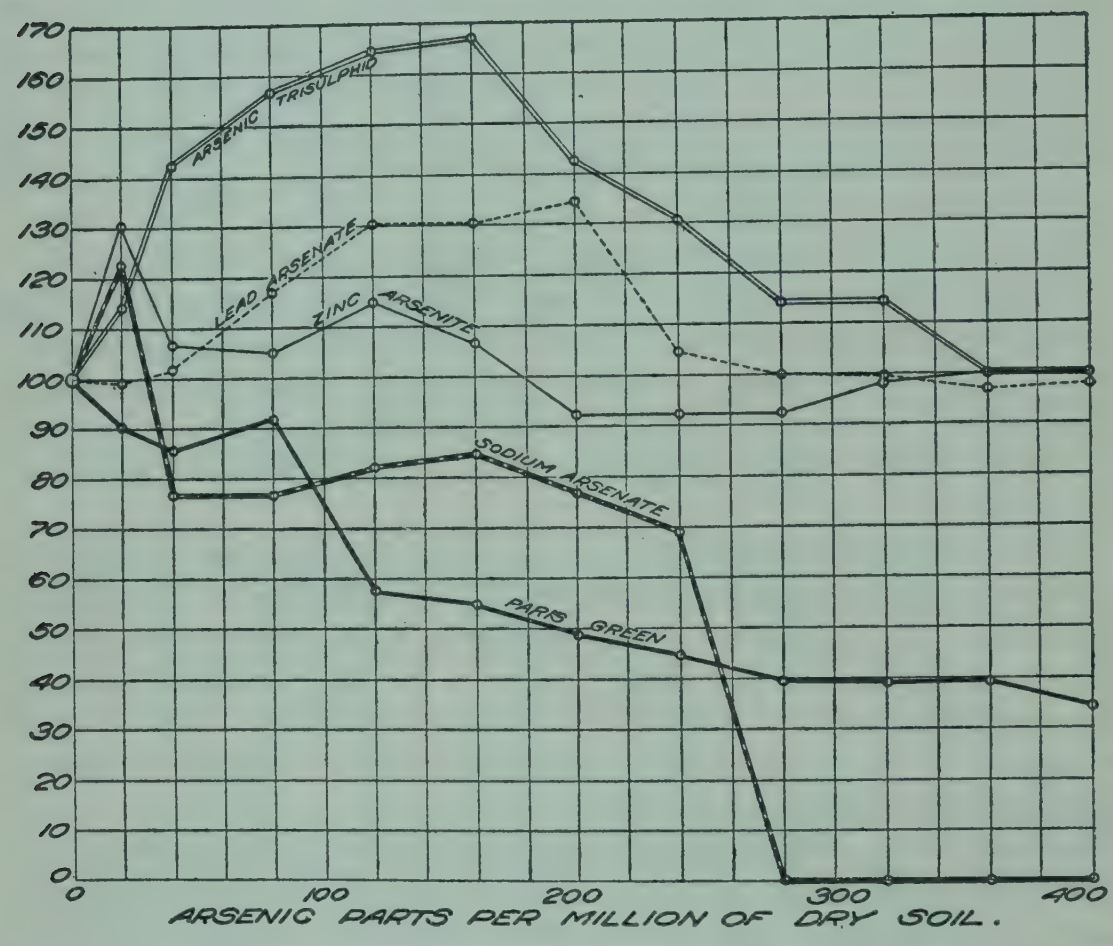


FIG. 1.—Graph showing the action of five compounds of arsenic on nitrogen fixation in dry soil. (Nitrogen fixed in untreated soil equals 100.)

stimulates growth much more in the nitrogen-fixing series than it does in the other series. The arsenic trisulphid has the greatest stimulating influence, followed in the order named by lead arsenate, zinc arsenite, and sodium arsenate. Paris green was the only compound tested which exerted no stimulating influence. It may be seen that the maximum stimulation was not obtained when equivalent quantities of arsenic in the various forms are applied to the soil. Hence, it seems possible that a relationship may exist among the various cases in the water-soluble arsenic found. In order to answer this, determinations were made of the water-soluble arsenic existing in the soil. The soil and arsenic,

together with 2 gm. of mannite, were placed in sterile tumblers, the water content made up to 18 per cent, and then incubated at 28° C. for 18 days. At the end of this period the soil was transferred by means of 1,000 c. c. of carbon-dioxid-free distilled water to large acid bottles. The mixture was left in these bottles, with occasional shaking, for 8 days, then filtered and the arsenic determined in an aliquot part (Greaves, 1913d). In another set the various forms of arsenic were mixed with 100-gm. portions of soil and 2 gm. of mannite and the water-soluble arsenic determined as above without incubation.

The results are given in Table II as milligrams of water-soluble arsenic occurring in 100 gm. of the soil both before and after the three weeks' incubation. Each reported result is the average of three or more closely agreeing determinations.

TABLE II.—*Quantity of water-soluble arsenic (in milligrams) in 100 gm. of soil before and after three weeks' incubation*

| Treatment. | Lead arsenate. | Arsenic trisulphid. | Sodium arsenate. |
|---|---|---|---|
| Arsenic added | 16.00 | 16.00 | 2.00 |
| Arsenic found before incubation | 1.04 | .14 | 1.08 |
| Arsenic found after incubation | 1.26 | 1.42 | 1.44 |
| Average | 1.15 | .78 | 1.26 |

The arsenic in each case became more soluble as bacterial activity progressed. This is especially marked in the soil containing arsenic trisulphid, which yielded 10 times the water-soluble arsenic after incubation that it did before. A remarkably close agreement is found to exist among the results obtained for water-soluble arsenic at the close of the incubation period, which shows that the maximum stimulating influence is obtained when soil contains between 10 and 15 p. p. m. of water-soluble arsenic. This is a quantity that exceeds that found in agricultural soil (Greaves, 1913b); hence, the influence of the arsenic occurring in soil must be to increase and not to retard nitrogen fixation. The maximum fixation varies with the form of arsenic applied. This is undoubtedly due, as will be pointed out later, to the elements accompanying the arsenic, which may have either a retarding or an accelerating influence upon the bacterial activity.

The finding of this marked stimulating influence of arsenic upon the nitrogen-fixing powers of soil raises a number of very interesting and important questions. Some of these are: (1) Does this stimulating influence exist in other soil or is there something inherent within this particular soil which makes its bacterial flora susceptible to the influence of arsenic? (2) Is the stimulating influence brought about by the retarding of injurious species or is it a direct stimulant to the soil organisms?

(3) Do the arsenic and arsenic compounds act as a source of energy to the nitrogen-fixing organisms or do they so influence the soil flora that it can utilize more economically the carbon compounds available? (4) What nitrogen-fixing organisms are there in the soil which are influenced by arsenic?

In order to find whether arsenic influences the nitrogen-fixing powers of other soils in a similar manner, three other soils were tested with and without arsenic. The soils vary greatly in chemical and physical composition. Soil A is a black loam of very light texture and, for an arid soil, high in nitrogen and humus. It is well supplied with phosphorus, potassium, and calcium carbonate and grew potatoes for 23 years. After this it was planted to oats for 2 years, and during the past 4 years has been planted in alfalfa. It has received some manure. Soil B is a sandy loam of much lighter color than soil A and contained much less humus and nitrogen, but an abundance of other elements. It has been cultivated for 28 years and during this time has been fallowed two summers. The remainder of the time it has been planted in wheat. Soil C is a heavy clay almost devoid of humus. The nitrogen is low, but the soil is well supplied with phosphorus, potassium, and calcium carbonate. While wet it is exceedingly sticky, and on drying it bakes like adobe. It has been tilled for 23 years, and during this time it has been fallowed for 3 years. The remainder of the time it has been in wheat. While it has received no manure during this time, it is still very productive. All of the soils are very fertile and well supplied with Azotobacter, and previous work has shown them to have high nitrogen-fixing powers.

The soils were all air-dried in the dark for 24 hours, ground in a mortar, sieved, weighed, and placed in sterile tumblers. Some were mixed with mannite and arsenic, others with mannite, while still others received only arsenic. They were all incubated in the regular manner, and the nitrogen determined as in the previous series. The results are given in Table III. Each reported result is the average of six closely agreeing determinations.

A marked stimulation is found in every case where the arsenic and mannite were applied to the soil, as compared with the results obtained where the mannite only was applied. The action of the various arsenical compounds follows the same order in each of these soils that it did in the first soil tested, being greatest with the lead arsenate and least with the sodium arsenate. The nitrogen fixed in the presence of arsenic but in the absence of mannite is usually considerably higher than that fixed in the presence of mannite and absence of arsenic. It would not be right to conclude from these results that the arsenic compounds furnish a source of energy to the nitrogen-fixing organisms, for these soils (Greaves, 1914, p. 456) have been found to fix appreciable quantities of nitrogen when incubated with an optimum moisture content without the addition of any carbon compound. It is likely that the arsenic makes the nitrogen-

gathering organism use more economically its usual source of carbon, which in the absence of mannite is probably the plant débris which has been slowly added to the soil. The belief that this is the case is strengthened by the fact that soil rich in organic matter (soil A) acts practically the same in the absence of mannite and presence of arsenic as it does when both arsenic and mannite are added to the soil. The clay soil (C), which is low in organic matter, acts about the same in the absence of arsenic as in the absence of mannite. It is interesting to note that in soils B and C the total fixation in the soil containing mannite plus that fixed by the soil containing arsenic approximates the total fixation in the series in which both arsenic and mannite are present.

TABLE III.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and without arsenic*

| Soil. | 16 mgm. of arsenic, 2 gm. of mannite, added to soil. | 16 mgm. of arsenic, no mannite, added to soil. | No arsenic, 2 gm. of mannite, added to soil. | Total of columns 2 and 3. |
|---|---|---|---|---|
| **LEAD ARSENATE** | | | | |
| A | 17.0 | 16.8 | 6.7 | 24.5 |
| B | 16.8 | 9.8 | 4.0 | 13.8 |
| C | 10.5 | 5.3 | 6.3 | 11.6 |
| Average | 14.7 | 10.6 | 6.0 | 16.6 |
| **ARSENIC TRISULPHID** | | | | |
| A | 16.3 | 15.6 | 13.8 | 29.4 |
| B | 12.6 | 7.0 | 7.6 | 14.6 |
| C | 10.6 | 5.6 | 4.2 | 9.8 |
| Average | 13.1 | 9.4 | 8.5 | 17.9 |
| **SODIUM ARSENATE** | | | | |
| A | 7.8 | 6.3 | 6.3 | 12.60 |
| B | 7.0 | 4.9 | 3.3 | 8.20 |
| C | 9.2 | 8.4 | 7.0 | 15.40 |
| Average | 8.0 | 6.5 | 5.5 | 12.0 |

In all of the tests so far reported the incubation period has been 18 days. Longer periods of incubation may give results very different from those so far obtained, for the stimulating influence of arsenic may be of short duration, and we may find later a slowing up of the reaction, or, inasmuch as we are dealing with the algebraic sum of many reactions which are taking place in the soil, we may find it to be negative. An attempt was made to determine this by the following experiment: 100-gm. portions of the high-humus soil (A) were mixed with 0.0728

gm. of lead arsenate and the moisture content made up to 18 per cent and then weighed. One-half of the samples thus prepared were sterilized in the autoclave and all of them placed in an incubator at a temperature of from 28° to 30° C. The moisture was made up weekly to its initial content. Beginning at the end of 20 days, six samples, three autoclaved and three not autoclaved, were used for the making of duplicate total-nitrogen determinations. The average excess of nitrogen in the unsterilized soil over that in the sterilized is given in Table IV.

TABLE IV.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil containing 0.0728 gm. of lead arsenate*

| Time incubated. | Nitrogen. | Time incubated. | Nitrogen. |
|---|---|---|---|
| *Days.* | *Mgm.* | *Days.* | *Mgm.* |
| 20 | 12.32 | 96 | 1.00 |
| 30 | —12.40 | 162 | 3.80 |
| 44 | —16.40 | 172 | 6.20 |
| 66 | —8.20 | | |

The greatest quantity of nitrogen was obtained at the end of 20 days. During the next 10 days, however, 24.72 mgm. of combined nitrogen disappeared. During the next 14 days there was a loss of only 4 mgm. From this time on there was a gradual increase in the amount of combined nitrogen found within the soil up to the end of the experiment, but even after 172 days' incubation there was less nitrogen in the soil than there was at the end of 20 days.

The great loss of nitrogen can not be entirely charged up to the arsenic added, for other workers (Ashby, 1907; Hoffmann and Hammer, 1910, p. 164) have noted, when working with impure cultures, a loss of nitrogen on prolonged incubation in the absence of arsenic. The loss is probably due to the soil's becoming compact, with the production of anaerobic conditions. This, assisted by the protozoa (Miller, 1914, p. 217), which appropriate too large a share of the limited supply of oxygen in the soil, prevents entirely the activity of the aerobic nitrogen-fixing organisms and greatly stimulates the activity of the denitrifying organisms of the soil. This can, however, only partly account for the phenomena; otherwise there would be a continual decrease in the nitrogen as the soil became more compact.

The fact that aeration plays a considerable part in the reaction is brought out by the following experiment, which differs from the preceding only in that the soil was thoroughly stirred, thus aerating it each time before making up the moisture content. The results of this experiment are given in Table V.

TABLE V.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of aerated soil with and without the addition of arsenic after different periods of incubation*

| Days incubated. | Nitrogen fixed in soil containing 0.0728 gm. of lead arsenate. | Nitrogen fixed in untreated soil. | Days incubated. | Nitrogen fixed in soil containing 0.0728 gm. of lead arsenate. | Nitrogen fixed in untreated soil. |
|---|---|---|---|---|---|
| 20 | 8.12 | 2.58 | 66 | 9.38 | 14.00 |
| 30 | 5.88 | 3.92 | 96 | 4.90 | 2.52 |
| 44 | 8.26 | 3.78 | 162 | 2.80 | 4.20 |

These results show conclusively that it was the lack of air in the former series which caused such great losses of nitrogen and that they could in no way be attributed to the arsenic added. This series was stirred but once a week and after the stirring the moisture content was made up to the optimum so that the soil became quite compact. It is quite likely that greater care in the aeration of the soil would have reduced very materially the loss of nitrogen which was observed in this series. In the first stages of the experiment the soil containing arsenic gained the greater quantity of nitrogen, while in the later stages the soils containing no arsenic were the highest. If, however, an average of the quantity found in each soil is taken, it will be found to be considerably higher in the soil containing arsenic than in the other.

It was thought that some of the questions referred to in the first part of this article could be answered more readily with the solution method than with soil. For this reason a series was incubated using a solution of the following composition:

Dibasic potassium phosphate ($K_2HPO_4$).... 0.2 gm.
Magnesium sulphate ($MgSO_4$)..... .2 gm.
Calcium chlorid ($CaCl_2$)..... .02 gm.
Ferric chlorid ($Fe_2Cl_6$)..... 1 drop (10 per cent solution).

This was made up to 1,000 c. c. with tap water and distributed in 100 c. c. portions into 750 c. c. Erlenmeyer flasks. One gm. of calcium carbonate was added to each, and the flasks were then sterilized and inoculated. One series was inoculated with *Azotobacter vinelandii*. This was done by making a suspension in sterile tap water of the organism and adding 5 c. c. of this suspension to each flask. In the other series the inoculating medium was 10 gm. of soil. The solutions were incubated at 28° to 30° C. for 18 days, and then the nitrogen determined in the manner previously outlined. The results are given in Table VI and are reported as milligrams of nitrogen fixed in 100 c. c. of the solution. Each reported result is the average of three closely agreeing determinations.

TABLE VI.—*Quantity of nitrogen (in milligrams) fixed in 100 c. c. of nutritive solution with and without the addition of arsenic*

| Treatment. | Inoculated with *Azotobacter vinelandii*. | Soil +0.0728 gm. of sterilized lead arsenate. | Soil +0.0728 gm. of unsterilized lead arsenate. |
|---|---|---|---|
| Nutritive solution + 1.5 gm. of mannite..... | 14.12 | 15.16 | 15.77 |
| Nutritive solution + 1.5 gm. of mannite and 0.0728 gm. of lead arsenate..... | 0 | 14.79 | 13.72 |
| Nutritive solution + 0.0728 gm. of lead arsenate..... | 0 | 1.45 | .52 |
| Nutritive solution + 1.5 gm. of mannite and 0.0272 gm. of arsenic trisulphid..... | .5 | 5.98 | 2.05 |
| Nutritive solution + 0.0272 gm. of arsenic trisulphid..... | 0 | .28 | .08 |

After the first series had been completed, it was thought possible that the heat in the autoclave had changed the solubility of the arsenical compounds and that this was the reason there was no fixation in the solution with arsenic. For this reason analyses were made of the soluble arsenic in 100 c. c. of the nutritive solution containing arsenic both before and after autoclaving. The determinations were made as previously outlined. The lead arsenate yielded 0.91 mgm. of soluble arsenic before autoclaving and 0.85 mgm. after autoclaving. The arsenic trisulphid yielded 0.40 mgm. before autoclaving and 0.42 mgm. after autoclaving.

The results indicate conclusively that the toxicity of the compound is not due to a difference in the solubility of the compound produced by the heat. In order to make sure of this, a series was arranged in which the arsenic was added just before inoculation and after the solution had been autoclaved. These results are given in the last column of Table VI and are slightly lower than those previously obtained with the arsenic. The *A. vinelandii* fixed no nitrogen in the presence of the arsenic. Even where the soil was used as the inoculating medium, the lead arsenate retarded nitrogen fixations to a certain extent. The toxic influence of the arsenic sulphid is very pronounced. These results show the care which must be used in drawing conclusions from the Remy-solution method as to what is to be expected in soils. They greatly strengthen the contention of Jönsson (1896) that the fact that Nobbe (1884) found arsenic solutions to be toxic to seedlings in water culture and concluded that arsenic, even in small quantities, is extremely toxic to plants does not indicate that these solutions will be toxic when in the soil. The results herein reported show arsenic to be extremely toxic to nitrogen-fixing organisms while in solution, but the same concentration in the soil is not only devoid of toxicity but acts as a powerful stimulant. This therefore establishes for the bacteria what Kanda (1904, p. 16) found to be true for the higher plants—namely, that dilute solutions of sub-

stances may be toxic when used in water culture, but that the same quantities when placed in the soil may act as stimulants.

The results reported for *A. vinelandii*, when considered in connection with those obtained for the soil, make very problematic the part played by Azotobacter, especially *A. vinelandii*, in these soils. The exact mode of action of the arsenic also remains a question. For these reasons the soil used in the first series was plated and the main nitrogen-fixing organisms isolated. Three types of Azotobacter were obtained. These have been designated Azotobacter A, Azotobacter B, and Azotobacter C. Azotobacter A has a nitrogen-fixing power of 6.86 mgm. of nitrogen per gram of mannite in Ashby solution, Azotobacter B a nitrogen-fixing power of 5.00 mgm., and Azotobacter C a nitrogen-fixing power of 6.44 mgm. of nitrogen.

The preceding results have shown that little information of value can be obtained by the solution method. Therefore another series was planned in which 100-gm. portions of the soil used in the first series were weighed into covered sterile tumblers and autoclaved at a temperature of 120° C. for 30 minutes, cooled, and the moisture content made up to 18 per cent. The soil was then inoculated with the various organisms which had been isolated. The soil portions were incubated for 18 days, the moisture content kept constant, and then the total nitrogen determined. Sterile blanks were incubated and analyzed as checks. Each reported result is the average of four or more closely agreeing determinations, so that the analytical error has been reduced to a minimum. The results are given in Table VII.

TABLE VII.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and without arsenic and inoculated with various nitrogen-fixing organisms*

| Inoculating organism. | Milligrams of nitrogen fixed in 100 gm. of soil treated with— | | |
|---|---|---|---|
| | 2 gm. of mannite, 0.0728 gm. of lead arsenate. | 2 gm. of mannite, no arsenic. | 0.0728 gm. of lead arsenate, no mannite. |
| Azotobacter A | 15.60 | 21.70 | 3.01 |
| Azotobacter B | 24.15 | 14.70 | 8.80 |
| Azotobacter C | 18.20 | 18.20 | 4.90 |
| Azotobacter A and B | 26.31 | 22.05 | 5.81 |
| Azotobacter A, B, and C | 18.40 | 17.70 | 6.65 |

The results reported above show for each organism a fixation much higher in the soil than was found in the solution. The results without arsenic, but with mannite, are as high as are reported in Table I with both mannite and arsenic combined, a fact which would seem to indicate that arsenic acts upon injurious species. This, however, does not account for the entire phenomenon, for we find in this series a very small fixation of nitrogen in the absence of mannite and presence of arsenic, while in

the ordinary soil with its mixed flora as great a fixation was obtained in the presence of arsenic as in the presence of only mannite. This probably indicates that some of the stimulation is due either to the fact that the arsenic acts upon allied species which are gathering carbon that can be used by the Azotobacter, or else to the fact that some species, possibly the cellulose ferments, are stimulated so that they render available to the Azotobacter the carbon-carrying compounds of the soil faster in the presence of arsenic than in its absence. Only one of the organisms isolated, Azotobacter B, is directly stimulated by arsenic. The stimulation, however, is very large in this case. It also fixes large quantities of nitrogen in the presence of arsenic and absence of mannite. These results are complicated by the carbonaceous material which occurs in the soil. For this reason a series similar to the above was incubated, using silica sand in place of the soil. The silica used was devoid of organic matter and had the following composition:

| | Per cent. |
|---|---|
| Silicon dioxid ($SiO_2$) | 97.5 |
| Ferrous oxid (FeO) | .1 |
| Aluminum oxid ($Al_2O_3$) | 1.7 |
| Calcium oxid (CaO) | .2 |

One-hundred gm. portions of this were sterilized in covered tumblers, and to each was added 1 gm. of calcium carbonate and 18 c. c. of sterile distilled water containing 0.02 gm. of potassium phosphate, 0.02 gm. of magnesium sulphate, and 0.002 gm. of calcium chlorid. The tumblers were inoculated with the various nitrogen-fixing organisms incubated with a constant moisture content at 28° C. for 18 days, and then the nitrogen determined as in the previous series. They were all compared with sterile blanks. The results are given in Table VIII as milligrams of nitrogen fixed in 100 gm. of sand. Each reported result is the average of six or more closely agreeing determinations.

TABLE VIII.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of quartz sand with and without arsenic*

| Inoculating material. | Sand and Ash-by solution, +0.0728 gm. of lead arsenate. | Sand and Ash-by solution, no arsenic. | Sand and Ash-by solution, no mannite, +0.0728 gm. of lead arsenate. |
|---|---|---|---|
| 10 c. c. of soil extract | 19.60 | 10.50 | 4.70 |
| Azotobacter A | 17.01 | 22.61 | 0 |
| Azotobacter B | 13.84 | 12.60 | 0 |
| Azotobacter C | 15.10 | 16.80 | 0 |

Qualitatively, the above results are the same as those obtained with the soil. Azotobacter B was the only one of the three organisms stimulated by the arsenic. Where the mixed flora were used, the stimulation was very marked, but the fixation in the absence of arsenic where either Azotobacter A or Azotobacter C was used is about the same as that

obtained in the presence of arsenic where the soil extract was used. This fact would seem to indicate that the main stimulation brought about by arsenic is due to its action upon injurious species. The results obtained in the presence of arsenic and absence of mannite indicate that the Azotobacter can not use the arsenic as a source of energy. The small fixation where the soil extract was used may be due to the nitrogen-fixing organisms obtaining a small quantity of carbon compounds from algæ which may have grown in the complex flora.

The results given in Table VII pointed strongly to the conclusion that the stimulating influence of the arsenic was due in part to an indirect action upon the nitrogen-fixing organisms, possibly an action which it exerts upon the cellulose ferment. A series was therefore arranged in which the cellulose ferments were used in connection with the Azotobacter.

In this series 100-gm. portions of the high humus soil (A) were placed in covered tumblers and sterilized in the autoclave and then treated as in Table IX. The Azotobacter was inoculated into 100 c. c. of Ashby solution. After three days the solution was thoroughly shaken and 5 c. c. of the solution were added to the sterile soil. The cellulose ferment was added by making a suspension of the organism in sterile distilled water and adding 5 c. c. of this to the soil. The moisture content was made up to 18 per cent and incubated for 18 days. Six samples of each were used, so that the results reported are the averages of six closely agreeing determinations. The results are given in Table IX. The cellulose ferments used were *Bacillus rossicus*, isolated by Kellerman, McBeth, and others (1913) from Geneva (N. Y.) soils, and *Pseudomonas effusa*, isolated by the same investigators from the soils used in this work.

TABLE IX.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and without arsenic in the presence and absence of cellulose ferments*

| Treatment. | Nitrogen gained. |
|---|---|
| *Azotobacter chroococcum* | 14. 70 |
| *Azotobacter chroococcum*, 0.0728 gm. lead arsenate | 14. 28 |
| *Azotobacter chroococcum*, *Bacillus rossicus* | 26. 18 |
| *Azotobacter chroococcum*, *Bacillus rossicus*, 0.0728 gm. of lead arsenate | 28. 00 |
| *Azotobacter chroococcum*, *Pseudomonas effusa* | 13. 30 |
| *Azotobacter chroococcum*, *Pseudomonas effusa*, 0.0728 gm. of lead arsenate | 22. 68 |
| Azotobacter B | 14. 46 |
| Azotobacter B, 0.0728 gm. of lead arsenate | 21. 00 |
| Azotobacter B, *Bacillus rossicus* | 15. 20 |
| Azotobacter B, *Bacillus rossicus*, 0.0728 gm. of lead arsenate | 19. 60 |
| Azotobacter B, *Pseudomonas effusa* | 14. 00 |
| Azotobacter B, *Pseudomonas effusa*, 0.0728 gm. of lead arsenate | 21. 00 |

In this series, as in the previous series in which *A. chroococcum* was used, it did not fix as much nitrogen in the presence of arsenic as it did in the absence of it. *A. chroococcum* fixes nearly twice the quantity in the

presence of *B. rossicus* as in its absence, and when arsenic is added to the two there is an even greater fixation. This is also the case with *P. effusa;* measured in terms of the increased nitrogen fixed by *A. chroococcum*, it may therefore be safely concluded that both of the cellulose ferments are stimulated by lead arsenate.

The Azotobacter B differs from the *A. chroococcum* in that it is directly stimulated by the arsenic, but is not as greatly helped by the cellulose ferment. In this case the lead arsenate greatly stimulates the activity of the cellulose ferments, and the stimulating influence is much greater with *P. effusa*, the normal habitat of which is this soil, than it is with *B. rossicus*. Hence, from this work it is safe to conclude that the cellulose organisms, so far as arsenic is concerned, obey the same laws as do the ammonifying, nitrifying, and nitrogen-fixing organisms of the soil.

It has been noted throughout all of this work that the soil taken direct from the field was stimulated to a much greater extent by the arsenical compounds than was the air-dried soil. Furthermore, it was noted that the soil which had stood in the laboratory for a great length of time was stimulated only very slightly by arsenic. For these reasons a series of experiments was planned to throw more light upon this substance or organism which disappears on drying.

Fred (1911) has suggested the use of filter paper for the separation of the protozoa. Later this has been shown by Kopeloff and others (1915) to be quite effective. Using this suggestion, 100-gm. portions of soil were placed in tumblers. To half of them was added 0.0728 gm. of lead arsenate, and the mixture was autoclaved until free from bacterial life. They were all inoculated with 10 c. c. of a solution obtained by shaking 100 gm. of soil in 1,000 c. c. of sterile water and then filtering through three thicknesses of a fine grade of quantitative filter paper, after which they were incubated and nitrogen determined as in the previous set. The results are given in Table X as milligrams of nitrogen per 100 gm. of soil. All results are averages of six determinations made on that number of incubated samples.

TABLE X.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of sterile soil inoculated with filtered soil extract, with and without arsenic*

| Time incubated. | 0.0728 gm. of lead arsenate. | No lead arsenate. |
|---|---|---|
| *Days.* | | |
| 20 | 16.10 | 14.70 |
| 30 | 3.08 | 2.52 |
| 44 | 2.80 | .30 |
| 66 | 1.94 | .14 |
| 96 | .28 | .28 |
| 162 | .78 | .85 |

It probably would have been better if in every case untreated soil could have been incubated with the variously treated soil, but this so greatly increased the number of determinations that it was not thought advisable. Furthermore, all the work has been done on the high-humus soil, A, without the addition of any carbohydrate, and repeated determinations have shown that the arsenic more than doubles the nitrogen fixed in the soil in 20 days, so that the absence of the stimulation can be safely attributed to the treatment. In the above results, it is readily seen that the soil extract on passing through filter paper loses to a very great extent its power of being stimulated by arsenic. Hence, it is safe to conclude that the main stimulating influence of arsenic upon nitrogen fixation is due to its suppressing something which is found in the soil and which is removed by the filter paper.

That this factor is to a great extent the same as is removed by heat is shown by the results reported in Table XI. The arrangement of this series of experiments was as follows: 100-gm. portions of the soil were weighed into covered tumblers. To one-half of the set was added arsenic—0.0728 gm. to each 100 gm. of soil. The tumblers were all carefully sterilized and half of them were placed in the incubator in the sterile condition. To the others was added a soil extract prepared by shaking one part of soil with two parts of sterile distilled water for three minutes. After standing for about five minutes the liquid was decanted and 10 c. c. portions of this were used to inoculate the soil. Before inoculating, this extract was placed in thin-walled test tubes in 10 c. c. portions and then held at the required temperature for exactly 15 minutes before adding to the soil. The moisture content was made up to 18 per cent and the whole incubated for 20 days. Each reported result is the average of six closely agreeing determinations.

TABLE XI.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil, with and without arsenic, inoculated with soil extract*

| Temperature of soil extract (°C.). | 0.0728 gm. of lead arsenate added. | No arsenic added. |
|---|---|---|
| Room | 8.77 | 5.11 |
| 50 | 9.24 | 9.00 |
| 55 | 14.28 | 14.14 |
| 60 | 12.60 | 16.38 |
| 65 | 13.85 | 14.42 |
| 70 | 12.18 | 13.02 |
| 75 | 12.88 | 11.34 |
| 80 | 13.44 | 12.66 |
| 85 | 11.54 | 10.36 |

The heating of the soil extract to a temperature of 55° C. for 15 minutes changes the soil so that it is no longer stimulated by arsenic. The heating of the soil extract to a higher temperature stimulates its nitrogen-

fixing properties. It is not, however, increased by the addition of arsenic. Hence, it would appear as if the substance which is suppressed by the arsenic is very thermolabile and is easily injured by drying, for it has been repeatedly brought to our attention that the drying of the soil prevents the arsenic from greatly stimulating its nitrogen-fixing properties. Harden and Young (1911, p. 72; 1906) have shown that the addition of arsenates to a yeast-juice sugar solution greatly accelerates the rate of fermentation of such a mixture. The close analogy existing between the chemical properties of phosphorus and arsenic led to the idea that possibly the arsenic replaced the phosphorus in the reaction characteristic of phosphorus, but they found that this is not the case, for while the arsenic has an optimum concentration, as has the phosphorus, there was no direct relationship between the amount of arsenate added and the extra amount of fermentation, the arsenic in this way acting more like a catalyzer than does the phosphorus. Furthermore it was shown that fermentation can not proceed in the absence of phosphorus, even though there be present either arsenates or arsenites. The arsenic acts mainly as a liberator of the phosphorus from the hexosephosphates and does not of itself enter into the vital reactions of the cell as does the phosphorus.

These facts make it likely that a similar action may be exerted by the arsenic upon the bacteria. For these reasons a series of experiments was arranged in which the phosphorus had been replaced by arsenic. These were carried on in the nitrogen-free quartz sand. To each 100 gm. of the sand there was added the quantity of carefully tested nutrient without phosphorus found in 100 c. c. of Ashby's solution. To one-half of them was added the phosphorus, while to the other half there was added 0.0728 gm. of lead arsenate. They were each inoculated with 1 c. c. of a soil extract and then incubated the regular length of time. The nitrogen determinations were made on them and sterile blanks with the following results: When incubated with complete Ashby's solution and 0.0728 gm. of lead arsenate, 100 gm. of sand fixed 11.62 mgm. of nitrogen. Similar samples without phosphorus but with arsenic fixed 0.03 mgm., while without phosphorus or arsenic there was fixed 0.01 mgm. of nitrogen. The results for the set with the complete nutritive media show that sufficient of the soil extract was taken to get the nitrogen-fixing organism, and the results without phosphorus show that there was not sufficient phosphorus in the 1 c. c. of soil extract to furnish phosphorus for the organisms. These results show conclusively that arsenic can not replace phosphorus in the vital activities of the nitrogen-fixing organisms of the soil, and establish for this set of organisms what Stoklasa (1897) has established for the higher phanerogams, Molisch (Lafar, 1911, p. 37) for algæ, Günther (1897) for the molds, and Harden and Young (1906) for the yeasts.

There is still the possibility that the arsenic liberates the phosphorus from its insoluble compounds in the soil and thus makes it more available to the micro-organisms. If this be the case, one would think that the addition of soluble phosphates to the soil investigated would increase its nitrogen-fixing powers. Experiments, however, did not bear out this assumption, for just as large a quantity of nitrogen was fixed in the absence of the soluble phosphate as in its presence. This was probably due to the fact that the soil under investigation was well supplied in the natural condition with soluble phosphorus. But that the arsenic did have an influence upon the solubility of the phosphorus of the soil was shown by the following experiment: 100-gm. portions of the soil were placed in covered tumblers. Of these, 24 received 0.0728 gm. of lead arsenate each, while the other 24 received none. The moisture was made up to 18 per cent and incubated for 20 days. At the end of this time the water-soluble phosphorus was determined in 12 of the treated and 12 of the untreated soils by extracting with 500 c. c. of distilled water and determining the phosphorus in the extract (Greaves, 1910). As an average of the 12 closely agreeing determinations of the soil treated with arsenic there was obtained 0.59 mgm. of water-soluble phosphorus, while the untreated soils yielded 0.52 mgm. This is a slightly greater quantity in the arsenic-treated soil than in the untreated, which is probably due to the fact that more of the phosphorus had been changed in the body of the soil organisms to nucleoproteins or phosphoproteins. That this is the correct interpretation is shown by the results obtained from the remaining samples. Twelve of these samples, six with and six without arsenic, were digested for six hours with 100 c. c. of 12 per cent hydrochloric acid and the phosphorus determined in the filtrate. The other samples were ignited and the phosphorus extracted by the 12 per cent hydrochloric acid determined. The average of the results thus obtained is given in the tabular form below:

| | |
|---|---|
| SAMPLES NOT IGNITED: | |
| Soil with arsenic | 105.6 mgm. of phosphorus. |
| Soil without arsenic | 100.0 mgm. of phosphorus. |
| Excess of acid-soluble phosphorus in soil with arsenic | 5.6 mgm. of phosphorus. |
| SAMPLES IGNITED: | |
| Soil with arsenic | 107.7 mgm. of phosphorus. |
| Soil without arsenic | 100.8 mgm. of phosphorus. |
| Excess of acid-soluble phosphorus in soil with arsenic | 6.9 mgm. of phosphorus. |

This would give by the Schmoeger method 2.10 mgm. of organic phosphorus in the arsenic-treated soil, while in the untreated soil there was

only 0.80 mgm. of organic phosphorus. This excess of organic phosphorus could not have come from the water-soluble phosphorus, as there was a difference of only 0.07 mgm. in the two soils; hence, it must be concluded that the arsenic increases the solubility of the phosphorus. This, however, may be due either to a direct interchange between the insoluble phosphorus of the soil and the arsenic or to its action upon bacteria, which causes them to become more active in growth and formation of various acids which act upon the insoluble phosphates of the soil, rendering them soluble.

## GENERAL CONSIDERATIONS

The data reported prove conclusively that the arsenical compounds, with the single exception of Paris green, stimulate the nitrogen-fixing organisms of the soil and that this influence varies qualitatively but not quantitatively with the various soils. The results also bring out the fact that both the anion and the cation of the compounds have a marked influence upon the growth of the organisms. With some compounds both the anion and cation act as stimulants, while with others one stimulates and the other is markedly toxic. It is likely that little or no influence is exerted upon the nitrogen-gathering organisms by the sodium (Lipman and Sharp, 1912), and that the stimulating influence noted with dilute solutions and the toxic influence exerted with more concentrated solutions are due entirely to the arsenic. It is quite likely that the stimulating influence which Rivière and Bailhache (1913) have found sodium arsenate to have upon wheat and oats is an indirect effect which is exerted upon the bacterial flora of the soil and which in turn influences the yield of the various grains.

Both the anion and cation undoubtedly act as stimulants in the lead arsenate. Stoklasa (1913) has shown that lead when present in soil stimulates the growth of higher plants. This he (1911) ascribes to the catalytic action of these elements on the chlorophyll. The results herein reported, together with those previously published (Greaves, 1913a), indicate that it is due to the influence of the compounds upon the biological transformation of the nitrogen in the soil. The fact that the lead plays no small part in the stimulating influence is borne out by the work of Lipman and Burgess (1914), who found lead to stimulate nitrifying organisms.

Paris green is toxic to the nitrogen-fixing organisms in the lowest concentration tested. This is due to the copper and not to the arsenic, as it is well known that the copper ion is a strong poison to many of the lower plants. Brenchley (1914) found it to be toxic to higher plants when present in water to the extent of 1 part in 4,000,000,000. Although Russell (1912, p. 47) states that it is not as toxic in soil as in water, Darbishire and Russell (1905) found it to be toxic in soils, and they failed to get a stimulating influence with it. Montemartini (1911)

has noted a stimulation with copper sulphate when used in dilute solutions. This, however, may have been due to the anion and not to the cation, as sulphates do stimulate plants by their action on insoluble constituents of the soil (Greaves, 1910, p. 298). The same interpretation could be placed upon the results obtained by Lipman and Wilson (1913) and also those reported by Voelcker (1913), in which they noted a stimulation with copper salts. Clark and Gage (1906) have found that very dilute solutions of copper have an invigorating influence upon bacterial activity. In order that the stimulation may be noted the copper must be present in small quantities. Jackson (1905) found that 1 part of copper sulphate in 50,000 parts of water killed *Bacillus coli* and *B. typhosus*. Kellerman and Beckwith (1907) found that the common saprophytic bacteria are more resistant to copper than is *B. coli*. There is considerable evidence (Lipman and Burgess, 1914; Greaves, 1913a, p. 8) that copper stimulates the ammonifying and nitrifying organisms of the soil, but these results show the nitrogen-fixing organisms of the soil to be very sensitive to copper, and if it does act as a stimulant it must be in extremely dilute solutions. The toxicity of the copper in the Paris green is great enough in the dilution of 10 parts in 1,000,000 to offset the great stimulating influence of the arsenic in combination with it.

The very marked stimulating influence noted where the arsenic trisulphid is used is very probably due to both the arsenic and the sulphur. Demolon (1913) attributed much of the fertilizing action of sulphur to its action upon bacteria, and Vogel (1914) found that sulphur decidedly increased the activity of the nitrogen-fixing organisms. The results which Russell and Hutchinson (1913, p. 173) obtained with calcium sulphid are interesting in this connection. They found that after 30 days there were five times as many organisms in the soil to which calcium sulphid had been added as in the untreated soil, and the yield of ammonia and nitrates in this time was one-third greater in the treated soil than in the untreated soil. This, in turn, reacts upon the crop harvested, as shown by Shedd (1914, p. 595).

The first part of the curve (fig. 1) for the zinc arsenite nearly coincides with that of the sodium arsenate, but the zinc arsenite stimulates in greater concentrations than does the sodium arsenate. This is partly due to the difference in solubility of the two compounds, but there is another factor which enters, and that is that the zinc also acts as a stimulant. Latham (1909) found that small quantities of zinc stimulated algæ. The same results have been obtained by Silberberg (1909) in working with higher plants. Ehrenberg (1910) concludes that zinc salts are always toxic when the action is simply on the plant, but that they may lead to increased growth through some indirect action on the soil. He found that zinc stimulated plant growth in soils, but when the soil was sterilized the zinc became toxic. Lipman and Burgess (1914, p. 133)

have shown that it does stimulate the nitrifying organisms and that the influence is shown by the yield obtained from such soils (Lipman and Wilson, 1913). The great variation in the results reported by the various investigators for zinc, arsenic, and lead is probably due to the fact that it modifies the bacterial flora of the soil, and when heated soil or water cultures are used a different result is noted. This, however, is not the only factor which enters, for these results show a marked difference in soil and in water. The lead arsenate stimulates the nitrogen-fixing organisms when placed in soils but becomes very toxic to the same organisms when placed in nutritive solutions.

The difference is due in part to the adsorption of the soil, but in this case we would have to attribute it to the silica compounds of the soil, for the nitrogen-fixing organisms are stimulated by arsenic in quartz sand

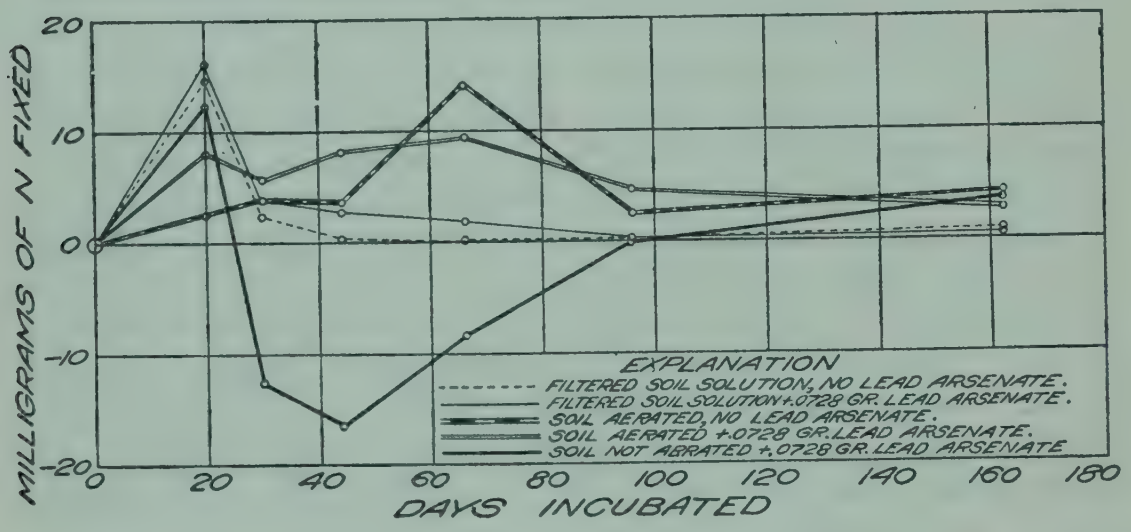


FIG. 2.—Graph showing the effect of aeration on the nitrogen-fixing activity of soil containing compounds of arsenic.

free from organic colloids. In this case the arsenic becomes concentrated at the surface, layers of the silica leaving the inner part of the water film comparatively free from arsenic, in which the micro-organisms multiply and carry on their metabolic processes. This being the case, one should, and probably could, find a water solution weak enough to stimulate bacteria. A great difference, however, between the solution and the sand-culture method is the greater aeration in the latter than in the former. That the aeration of a cultural medium does play a great part in determining the activity of the nitrogen-fixing powers of a soil is very strikingly brought out in figure 2. The graphs in this figure are made from the data given in Tables IV, V, and X.

It is remarkable how the aeration of the soil or the filtering of the soil extract can prevent the great loss of nitrogen which is noted at first in the unaerated soil. This can not be attributed directly to the denitrifying organisms; otherwise it would not be removed by filtration. The graphs

also bring out the fact that the addition of arsenic and the filtering of the soil only shift for the time the equilibrium within the soil, and later it tends to regain its old equilibrium. This is a condition which coincides well with what one would expect if the limiting element were some other microscopic forms of life. The filter would not separate them quantitatively, and it is possible that the arsenic has only a selective influence. Later, many of the organisms become accustomed to its presence; or, what is more likely, the arsenic becomes fixed (McGeorge, 1915) within the soil.

That this limiting factor is a thermolabile body is brought out more clearly in figure 3, which is made from the data reported in Table XI.

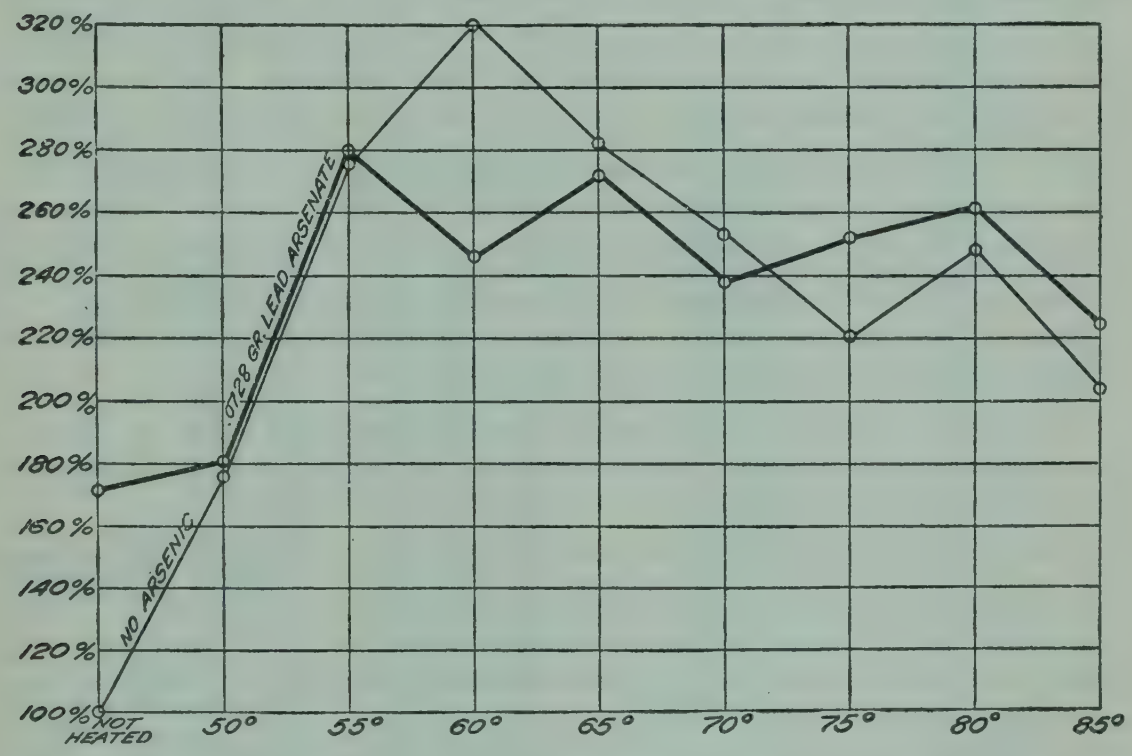


FIG. 3.—Graph showing the effect of heat on the nitrogen-fixing power of soil treated and not treated with arsenic.

The quantity of nitrogen fixed by the unheated soil receiving no arsenic has been taken as 100 per cent, and the heated soil with and without arsenic is compared with this.

The heating of the soil extract to 50° C. for 15 minutes has exactly the same influence measured in terms of nitrogen fixed as does 0.0728 gram of lead arsenate. The stimulating influence of heat is noted even in the presence of arsenic and reaches its maximum effect in the absence of arsenic at 60°, while in the presence of arsenic at 65° above these temperatures there is a decline in the nitrogen fixed. But even the soil inoculated with solutions which had been heated to a temperature of 85° fixed nitrogen; or at least there is more nitrogen accumulated in such soil than in that inoculated with the untreated soil solution. The results indicate that many of the organisms which take part in the gathering of nitro-

gen in this soil are very resistant to heat. It is also significant that the greatest stimulating influence is exerted in soil which had been inoculated with solutions heated just above what Cunningham and Löhnis (1914) found to be the thermal death point of soil protozoa.

The data presented in this paper, together with these presented in former publications, make it possible to compare the sensitiveness of the ammonifying, nitrifying, and nitrogen-fixing organisms toward the various arsenical compounds. Figure 4 represents the percentage of activity of the various classes of organisms in the presence of 400 p. p. m. of arsenic in the form of the various arsenical compounds. The untreated soil has been taken in every case as 100. The ammonifying organisms are retarded more by the lead arsenate than the nitrogen-fixing or nitrifying organisms. The latter two are influenced in nearly the same way by this concentration of lead arsenate. All three types of organisms are influenced in the same order by the arsenic trisulphid, while with the zinc arsenite the nitrogen-fixing and nitrifying organisms act about normally in concentrations of 400 p. p. m. of arsenic, but the ammonifiers are greatly depressed. Paris green stimulates the nitrifiers, but greatly depresses the other types of organisms. The results, with the exception of copper, show that the nitrifying and nitrogen-fixing organisms are very similar.

In figure 5 are shown graphically the quantities of arsenic in the form of various arsenicals which are required by the different organisms to give the greatest stimulation.

It has been shown that stimulation within a specific group of organisms varies with the quantity of water-soluble arsenic and the stimulating influence of the electropositive ion associated with the arsenic. But when we examine stimulation by these substances with different groups of organisms, we find a marked difference which can not be attributed to solubility but must be due to a physiological difference existing in the various organisms; for instance, the nitrogen-fixing organisms require 200 p. p. m. of arsenic in the form of lead arsenate for the greatest stimulation, while the nitrifiers and ammonifiers require much smaller quantities. For maximum stimulation with arsenic trisulphid the nitrogen-fixing organisms require the greatest concentration, followed by the nitrifying and ammonifying organisms in the order given. Zinc arsenite, on the other hand, has to be present in large quantities for a maximum stimulation of the nitrifying organisms, while very small quantities give a maximum stimulation with the other two groups of organisms. Practically the same order is followed by the organisms in the presence of sodium arsenate and Paris green, there being, however, this significant difference, that neither the ammonifiers nor the nitrogen-fixing organisms are stimulated in any concentration by the presence of copper, and it is quite possible that the same holds for the nitrifying

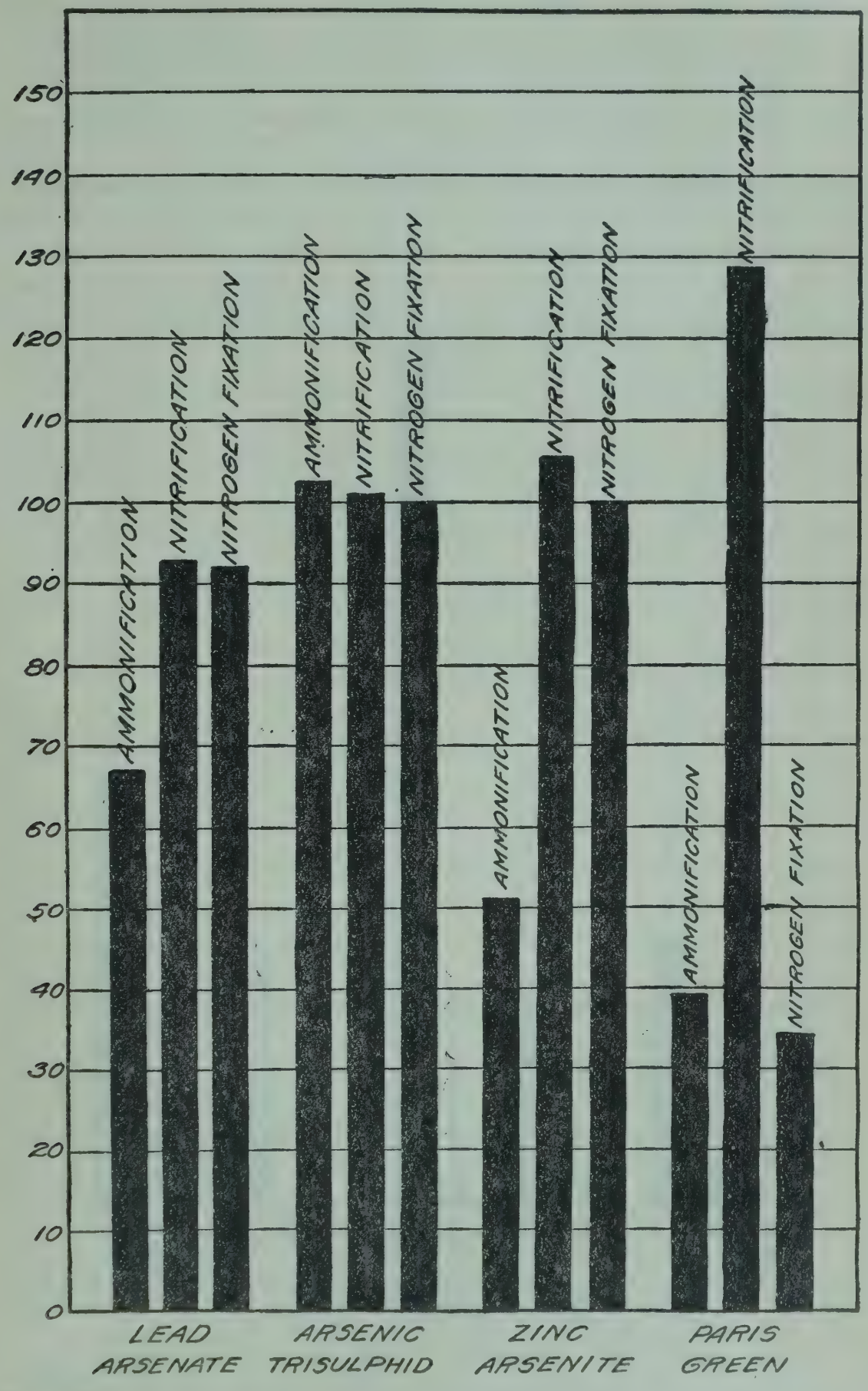


FIG. 4.—Effect of various arsenic compounds in the ratio of 400 parts of the compound to 1,000,000 parts of soil on the activity of various soil organisms.

organism. This set of organisms are, however, more resistant to copper than are others, and what we have occurring is a suppression of other types which feed on nitrates, thus permitting a greater accumulation

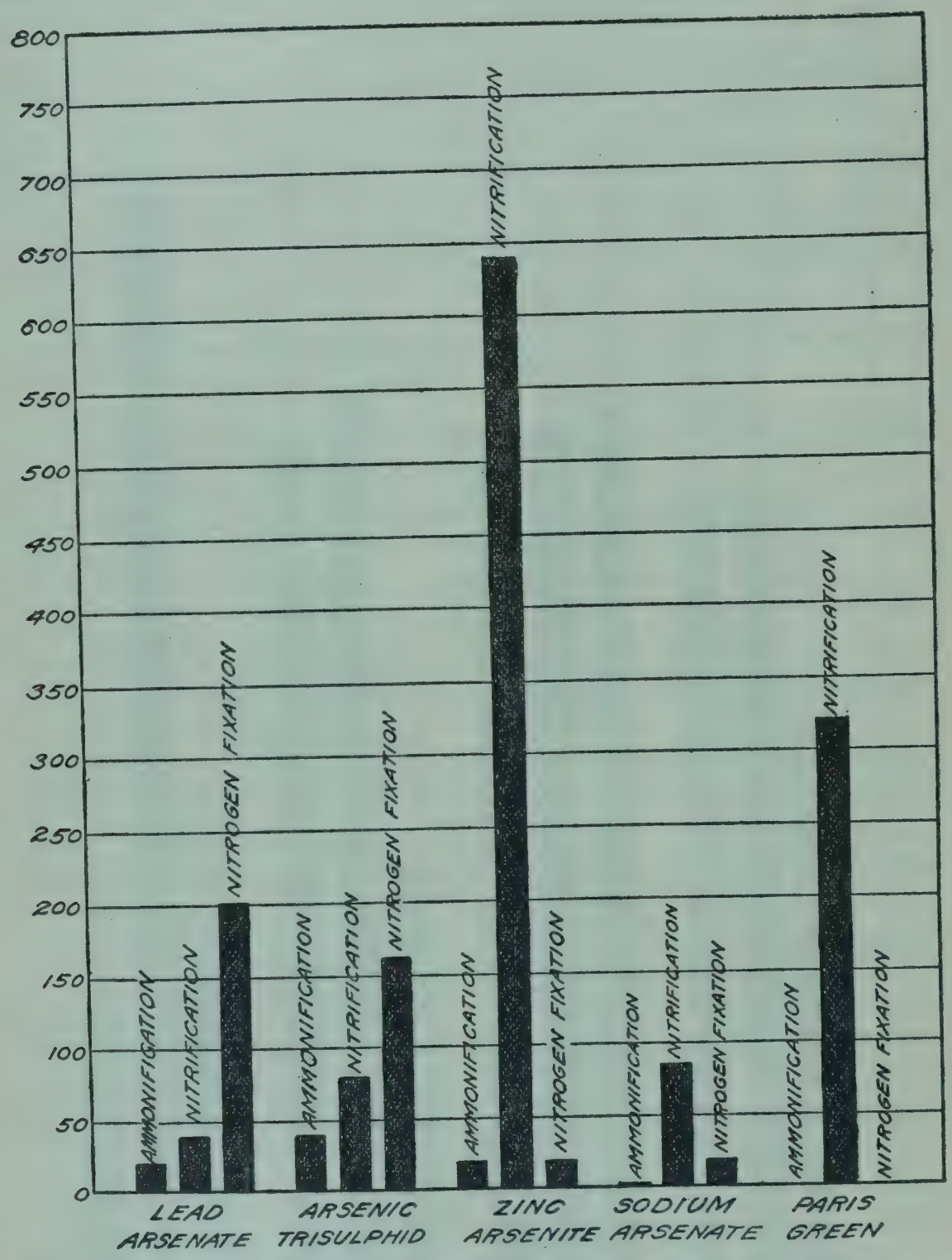


FIG. 5.—Graph showing parts per million of various arsenic compounds in the soil at which the greatest stimulation occurred.

of nitrates under these conditions. While not so likely in the other cases, the same possibility does arise. This, however, can be answered definitely only by further experiments.

SUMMARY

Arsenic, when applied to a soil in the form of lead arsenate, sodium arsenate, arsenic trisulphid, or zinc arsenite, stimulates the nitrogen-fixing powers of the soil. This stimulation is greatest when lead arsenate is applied and least when zinc arsenite is applied. Paris green did not stimulate in any of the concentrations. This compound becomes very toxic when the concentration reaches 120 p. p. m. The toxicity of this compound is due to the copper and not to the arsenic contained in it. Sodium arsenate became toxic when a concentration of 40 p. p. m. of arsenic was added, and when 250 p. p. m. were added it entirely stopped nitrogen fixation. Lead arsenate was not toxic even at a concentration of 400 p. p. m. of arsenic. The toxicity of arsenic trisulphid and zinc arsenite was very small at this concentration.

The stimulation noted when arsenic is added to a soil is not due to any inherent peculiarity of the soil used, for soils which vary greatly in physical and chemical properties had their nitrogen-fixing powers greatly increased when arsenic was applied to them. Soils high in organic matter fixed as much nitrogen in the presence of arsenic and in the absence of mannite as they did in the presence of mannite and absence of arsenic. The stimulation is greatest when the water-soluble arsenic content of the soil is about 10 p. p. m. This quantity exceeds that found in most soils, so it is likely that in agricultural practice arsenic will stimulate and not retard bacterial activity in the soil.

Only one type of Azotobacter was isolated which was stimulated by arsenic, and in this case the stimulation was due to the organism utilizing more economically in the presence of arsenic its source of carbon than it did in the absence of arsenic. The arsenic compounds do not act as a source of energy to the organisms. The main part of the stimulation noted in the soil with its mixed flora is undoubtedly due to the arsenic inhibiting injurious species.

A quantity of arsenic which acts as a stimulant to bacteria when placed in soil may become very toxic when tested by the Remy-solution method.

Arsenic can not replace phosphorus in the vital process of the nitrogen-fixing organisms, but it can in some manner liberate the phosphorus from its insoluble compounds. This may be either a direct or an indirect action.

Arsenic stimulates the cellulose ferments, and these in turn react upon the activity of the nitrogen-fixing organisms.

The nitrogen-fixing powers of soil extract, of filtered soil extract, and soil dried for some time are only slightly stimulated by arsenic, showing that arsenic acts mainly by the removal of a thermolabile body which occurs in the soil.

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# TRANSMISSION AND CONTROL OF BACTERIAL WILT OF CUCURBITS[1]

By FREDERICK V. RAND, *Assistant Pathologist*, and ELLA M. A. ENLOWS, *Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry*

## WILT TRANSMISSION

That the striped cucumber beetle (*Diabrotica vittata* Fab.) is a direct carrier of the bacterial-wilt organism (*Bacillus tracheiphilus*) from infected to healthy cucurbits was shown several years ago by Smith.[2] He also expressed the conviction that it was the most important, if not the only, summer carrier, and stated the possibility of its serving also as the winter carrier of the disease. Observation and experiment by the senior writer[3] during the last two seasons have abundantly confirmed the implication of the striped cucumber beetle as a summer carrier and have brought out strong proof that this insect is not only the principal summer carrier but also the winter carrier of the wilt organism. The twelve-spotted cucumber beetle (*D. duodecimpunctata* L.) must be included with the striped cucumber beetle at least as an important summer carrier of the disease.

## INSECT TRANSMISSION

Relative to cucumber beetles as winter carriers, several direct cold-storage tests have been carried out by the writers in Washington. During the summer and fall of 1915 hundreds of beetles were collected and placed in cold storage at temperatures ranging from 4° to 10° C. These early experiments were conducted partly with a view to determining the proper conditions of feeding prior to storage and the temperature and humidity most favorable to hibernation in storage. The optimum environment for hibernation varies for different insects, and it is necessary to work out this problem for each species. Consequently in these preliminary tests the greater portion of the beetles placed in cold storage was lost. Infection experiments with the few surviving beetles gave the results here detailed.

EXPERIMENT 1.—Several striped cucumber beetles were collected in October, 1914, and fed about two weeks on cucumber vines (*Cucumis sativus*) wilting as a result of natural infection with *B. tracheiphilus*. After six weeks' hibernation in cold storage the five surviving beetles were caged with a young squash plant on which

[1] Some of the details of the field experiments at East Marion, N. Y., were carried out by Mr. Wayland C. Brown, of the Bureau of Plant Industry. The land used in these experiments was furnished by Messrs. J. H. Douglass and G. S. Nowell, of East Marion.

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they were allowed to feed for 11 days. Observation after two weeks showed unmistakable signs of incipient wilt around some of the beetle injuries on the leaves—that is, a lighter dull green and slight flaccidity of the tissues. With the expectation that the wilt would extend throughout the leaves the pouring of plates was deferred. However, these incipient infection areas dried up without spreading further, and consequently it was impossible to obtain cultures. That *B. tracheiphilus* was present in the wilted vines fed to these beetles was shown by the subsequent inoculation of cucumbers, cantaloupes, and squashes with cultures obtained from these wilted vines (strains R230 and R235). Numerous inoculations with these two strains have shown them to be virulent upon cucumbers and cantaloupes, but inoculations on several varieties of squash have given nothing more than incipient infection.

EXPERIMENT 2.—On October 25, 1915, striped cucumber beetles were collected at Giesboro Point, D. C., in a squash field where bacterial wilt was very prevalent. These beetles were fed for three days on plants which were wilting as a result of inoculation with pure cultures of *B. tracheiphilus*. They were then placed in small boxes provided with screened covers, and held in the ice compartment of a refrigerator at a temperature of about 10° C. for five weeks and four days. At the end of this time (Dec. 6) the beetles were removed and placed in cages containing young cucumber plants. Four to six beetles were placed in each of the six cages used, each cage containing three young plants. After being allowed to feed on these plants for 10 days the beetles were removed and the plants kept in one of the Department greenhouses where there had been no cucurbit wilt since the preceding spring and where no cucurbitaceous insects were present.

On December 17 leaves injured by the beetles on three of these plants were wilted. Microscopic examination showed bacteria present in great number in the vessels of the petioles, and poured plates from the wilted leaves and petioles gave pure cultures of the wilt organism (strain R313). Needle-prick inoculations from these cultures again gave typical wilt on cucumber plants. On December 24 a gnawed leaf on a fourth plant was found wilting, and was removed from the plant. Enormous numbers of bacteria were present in the vascular tissues, and cultures (strains R315 and En126) isolated therefrom gave also successful infection when pricked into the leaves of young cucumber plants. From the portion of petiole remaining the wilt gradually extended throughout the plant, which finally collapsed. On January 4 another plant was found entirely wilted. The gnawed leaf which had wilted first, and from which the wilt had spread throughout the plant, was photographed and preserved. Cultures (strain En124) and paraffin sections (En36) were made from the petiole of this leaf. The organism isolated gave typical infections when inoculated into cucumber plants.

EXPERIMENT 3.—Another lot of *D. vittata* collected in the squash field referred to in experiment 2 was fed for three days on old wilting stems of squash (*C. maxima*) collected in the same field. After keeping these beetles in storage for two months under the same conditions as in experiment 2, they were removed and caged for five days with 12 young cucumber plants. Although these plants were under observation for over two months no wilt appeared in any of them.

EXPERIMENT 4.—On December 16, 1915, five specimens of *D. vittata* and four of *D. duodecimpunctata* hibernating under natural conditions in the squash field at Giesboro Point, D. C., were sifted from the surface soil and taken to the greenhouse. The striped and spotted beetles were placed at once in separate cages, each containing three young cucumber plants. Although the beetles fed freely on these plants, the results of this experiment were negative.

The negative results in experiment 3 possibly may be explained by the fact that the wilted plants fed to the beetles were old, ripe squash

vines which had been diseased for a long time. Doubtless few living organisms were present, since great difficulty was experienced in obtaining cultures of *B. tracheiphilus* from this field (strains En102 and En110). The beetles used in experiment 4 were collected when hibernating in a field where wilt was known to have occurred, but it is evidently not possible to determine whether they had fed upon wilted plants. On the other hand, it is not reasonable to assume that all beetles which have fed upon wilted plants would necessarily be able to carry infection on their mouth parts for any great length of time. Experiments 1 and 2 show that at least in some cases the striped beetles may carry the wilt organism for at least five or six weeks and still be able to infect healthy plants. This, in connection with the field experiments previously published,[1] seems to establish beyond doubt that *D. vittata* is a winter carrier of the cucurbit organism.[2] Experiments with other species of insects have thus far given negative results, as here detailed.

In each of seven tests carried out with the common squash bug (*Anasa tristis* DeG.) during the summer and fall of 1915 in field and greenhouse, two to six of these insects were fed for one to three days on wilted cucumber leaves and petioles and then inclosed with several healthy cucumber plants. After feeding on these plants for one to two days the bugs were removed and the plants kept under observation for three to four weeks. No wilt appeared in any of these plants, but no absolute conclusion can be drawn from the negative results of so small a series of tests.

The twelve-spotted (or squash) lady beetle (*Epilachna borealis* Fab.) was very scarce in eastern Long Island during the season of 1915, but two tests with it similar to those outlined above gave negative results.

The melon aphis (*Aphis gossypii* Glov.) and the flea beetle (*Crepidodera cucumeris*) apparently do not serve as wilt carriers. This has been shown by the negative results from transfer of insects fed upon wilted plants to healthy cucumber plants in insect-proof cages (three tests), and by the fact that no wilt developed during the season in cucumber plants grown in 48 large screened cages (East Marion, Long Island, N. Y., 1915), although numerous wilted plants occurred around all of these cages, and aphids and flea beetles had free access through the meshes of wire netting and were abundant both outside and inside the cages.

In only 2 out of 50 cages did wilt appear and in these cases striped cucumber beetles had gained access or had been purposely introduced, and the disease had started from points gnawed by the beetles.

[1] Rand, F. V. Op. cit.

[2] Wild cucurbits may be eliminated as possible carriers of bacterial wilt so far as the experiments at East Marion are concerned. Personal observations, together with those of Burnham and Latham (Burnham, Stewart H., and Latham, Roy A. The flora of the town of Southold, Long Island and Gardiner's Island. *In* Torreya, vol. 14, nos. 11–12, 1914), and a search through the herbaria of the New York and Brooklyn Botanical Gardens, have established beyond doubt that no wild Cucurbitaceae occur within 10 to 15 miles of the experimental plots.

In each of eight direct summer field tests, one to five striped cucumber beetles were fed for one to three days on wilting cucumber leaves and petioles and then at once caged up with several healthy young cucumber plants. In six out of these eight tests bacterial wilt appeared in one to two weeks and only on plants gnawed by the beetles.

In the two fields (East Marion, Long Island, N. Y.) where spray tests were carried out during the season of 1915 the prevalence of bacterial wilt closely followed that of the striped cucumber beetle. Throughout the season careful and frequent observation failed to disclose a single case of wilt which had not evidently started in a part of the plant injured by cucumber beetles (Pl. LIII). In these two fields no wilt had appeared up to the 1st of July. A few cases were observed on July 3, while the greatest number of cases was found during the last 10 days of the month. Practically no new cases of wilt appeared after the 30th of July. The first striped cucumber beetles of the season were seen from June 15 to 17. In field 1 the first beetles were found on June 17 between cages 14 and 15.[1] On July 3 there were only seven cases of wilt in the whole field, and six of these occurred near or about where these beetles had been collected. The beetles were most numerous between June 24 and July 8, in fact so numerous that in order to save the plants from entire destruction an application of a proprietary dust insecticide (containing lime, Paris green, etc.) was made upon the unsprayed plots. Thus, for a few days, or until new growth appeared on the vines, there were no untreated cucumber plants in these two fields upon which the beetles could feed. From this date on, the beetles began to disappear from these fields. In the variety-test block and commercial fields in the vicinity the plants were younger and for the most part were untreated. In fact, most commercial plantings were just breaking through the ground on July 10. Such fields present an attractive feeding ground for the beetles. In the two experimental fields there were only a few beetles present on July 15, and they were exceedingly scarce after July 30.

When it is remembered that under field conditions usually one to three weeks elapse between time of infection and the appearance of wilting in the plants, it will be seen that the rise and fall in the number of plants with bacterial wilt closely follows the rise and fall in the number of beetles (fig. 1).

The two fields just discussed had been planted to cucumbers the preceding season. About a quarter of a mile from field 1 a cucurbit variety test block was located. This land had not been plowed for several years. Although separated only by slightly rolling, plowed land from field 1, where striped cucumber beetles appeared on June 17, no beetles appeared here until about the end of the first week in July. This was just after

[1] These beetles were used in the cage transmission tests recorded in a former paper (Rand, F. V., op. cit.) and mentioned in a preceding paragraph.

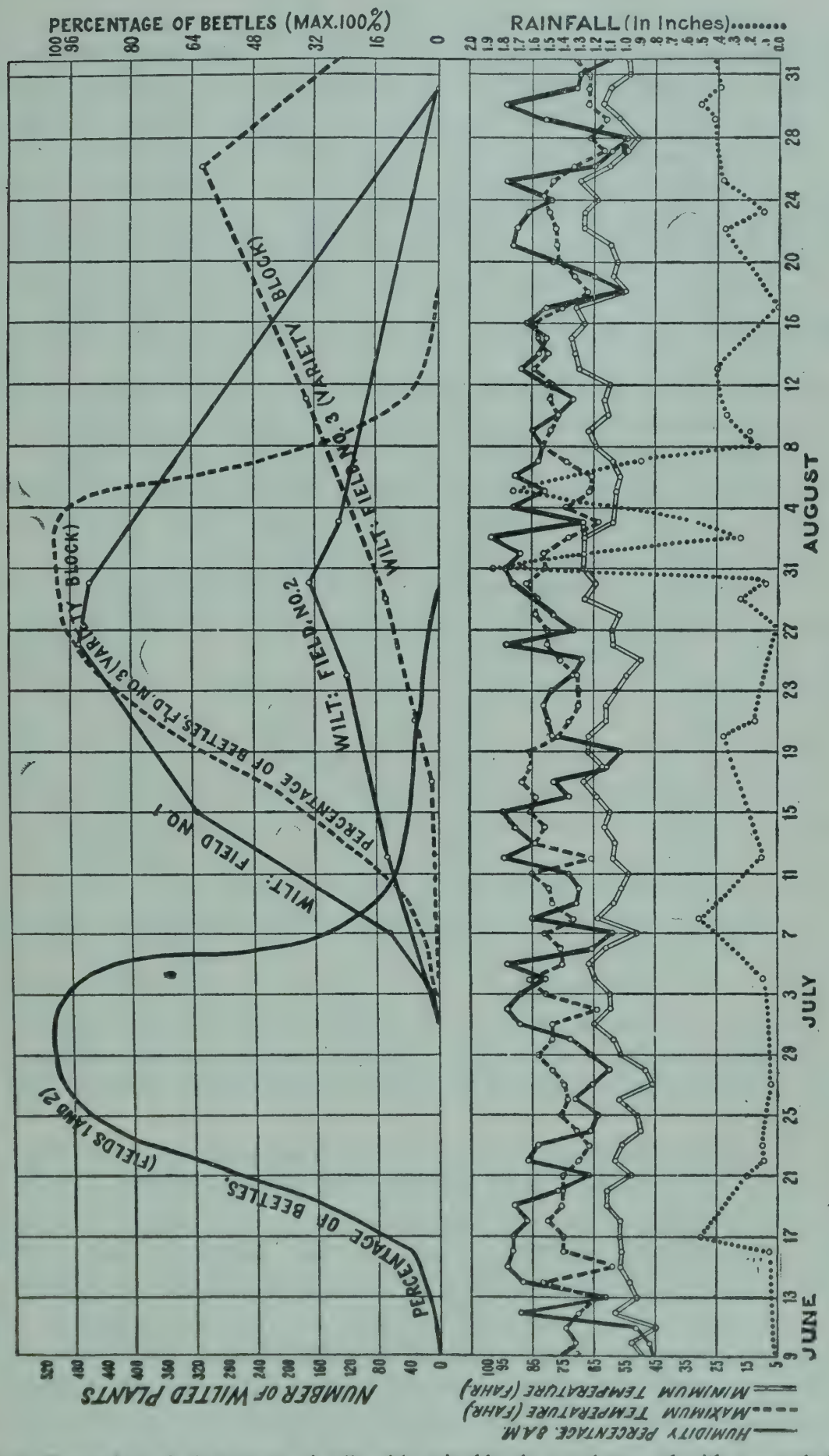


FIG. 1.—Comparison of the amount of wilt with striped-beetle prevalence and with meteorological phenomena in three fields, East Marion, Long Island, N. Y., season of 1915.

they had begun to disappear from field 1. In the variety test the first scattered cases of wilt were observed on July 17, whereas in field 1 the first cases were noted on July 3. The largest number of cases of wilt in the variety block were found between August 15 and 30, and the striped cucumber beetles were most numerous here during the last part of July. Again, allowing for the necessary time between infection and actual wilting, it will be noted that here also there is a direct relation between the number of wilt infections and the number of beetles present (fig. 1).

The graphs (fig. 1) show the daily relation between meteorological conditions, the number of beetles present, and the number of wilted plants in the three fields from June 10 to August 31. In these graphs there is shown a definite relation between the beetle and the wilt curves, but no relation between the latter and the meteorological curves. The meteorological instruments from which the data were obtained for this graph were kept in a United States Weather Bureau instrument shelter at ground level, so that the environment would correspond as nearly as possible to that of the cucumber plants (Pl. LIV, fig. 4).

Reference should be made to the fact that in taking notes the total number of plants showing bacterial wilt was recorded at each date of observation. This number included not only the new cases but also cases holding over from the preceding observation. Ordinarily the older the plant at the time of infection the longer the interval between infection and death. This explains the apparently too great interval between the maxima of the beetle and wilt curves. If it had been the original intention to represent graphically the relation between the prevalence of the beetles and the occurrence of wilt, the data would have been obtained in a form better suited to this method. It was only after tabulating the results of the field observations that the very striking parallel was noted. Obviously it would be impossible to enumerate absolutely the beetles present in a field; hence, the percentages used in the graphs are based partly on actual counts and partly on careful estimates made throughout the season. In the curves, 100 per cent represents the maximum number of striped cucumber beetles present at any one time.

Attention should be drawn to the fact that although there was a difference of only three days in planting time between field 1 and field 3, the beetles appeared between two and three weeks earlier in field 1, which had been planted to cucumbers the preceding season. This would suggest that these insects hibernated in or near the old cucumber field and that they did not leave this field the following spring as long as young and tender plants remained for them to feed upon. A similar tendency of both striped and twelve-spotted cucumber beetles to hibernate in old cucurbit fields was observed by the writers near Congress Heights, D. C. The first frosts occurred in these fields during the first part of October. About the middle of December, 1915, soil siftings to

a depth of 7 inches were made at numerous points over this squash field. Considerable numbers of dormant beetles were found under clods, old vines, mummied squashes, and around the bases of old squash stems just beneath the soil surface. No beetles were found below the first 2 inches and most of them were found at a depth of less than 1 inch.

### SOIL TRANSMISSION

In the experiments of 1915 at East Marion, Long Island, N. Y.,[1] bacterial wilt was not transmitted to the plants from the soil, although in the same fields during the preceding season the crop had been largely destroyed by this disease. In a large number of greenhouse inoculations into one of two or more cucurbit plants in a single pot (seven experiments, including in all 126 pots), none but the inoculated plants ever took the disease, although the latter wilted to the ground, and the pots were kept under observation from one to three months. The house was free from cucurbitaceous insects.

In addition to these observations and experiments relative to soil transmission three series of direct soil inoculations were made:

SERIES OF MARCH 18, 1915.—Thirty-two Arlington White Spine forcing cucumber plants 4 to 5 inches high, transplanted March 9 and not disturbed from that date until the date of inoculation, were inoculated as follows:

Eight cucumber plants not root-pruned and the same number of plants root-pruned were inoculated with strain R230 by pouring on the soil beef-bouillon cultures 6 days old. Sixteen plants were inoculated in the same way with strain R235. Sixteen plants were root-pruned and the soil moistened with tap water only, these plants being held as checks. The cultures used were tested as to virulence by needle-puncture inoculations into the leaves of several cucumber plants of the same age and variety.

All plants inoculated by needle puncture promptly wilted.

On April 1 the 16 inoculated plants which had been root-pruned showed two cases of wilt. No wilt was evident in the 16 non-root-pruned plants at this date.

On April 12, among the 16 root-pruned plants there were 10 wilted and among the 16 non-root-pruned there were 2 wilted. The 16 check plants (root-pruned) showed no signs of wilt.

Isolations were made from all plants showing infection from the soil, and these cultures produced wilt promptly upon inoculation into leaves of healthy plants.

The experiment was continued for two months from the date of inoculation, but no further cases of wilt appeared.

SERIES OF MARCH 31, 1916.—Sixty Chicago Pickling cucumbers planted January 28, 1916 (transplanted once), in pots in the greenhouse

[1] Rand, F. V., 1915. Op. cit.

were inoculated by pouring on the soil tap-water suspensions of *B. tracheiphilus* from beef-agar slants 6 days old. Of these 60 plants 24 were root-pruned, and the remaining 36 were left uninjured. In this experiment 26 strains, isolated from squash, cucumber, and cantaloupe (*Cucumis melo*), were used, and each culture was proved to be virulent by needle-puncture inoculation into the leaves of healthy cucumbers of the same age and variety. The virulence tests were made about 30 minutes before these agar slants were used for the soil inoculations.

The plants were under daily observation, and there were no signs of wilt until April 11, when one of the root-pruned plants wilted. Between this date and April 19, 6 of the 24 root-pruned plants (25 per cent) and 8 of the 36 uninjured plants (22 per cent) wilted. Examination of the stems and main roots showed the typical stringy slime in the vascular system, and cultures from these roots proved the presence of *B. tracheiphilus*.

It will be seen that in this test the percentage of wilt was about the same in the root-pruned plants and in those not root-pruned. However, too much weight can not be given to the results of this experiment, since the cucumbers had been recently transplanted and examination of the roots showed considerable eelworm injury. These wounds might, of course, afford entrance for the wilt organism.

## SEED TRANSMISSION

Ripe cucumber fruits were collected from wilted vines at Malone and Constable, N. Y., on September 23, 1914. Five of the fruits from Malone and one from Constable showed on cutting an abundance of the sticky white ooze characteristic of this bacterial wilt, and microscopic examination revealed enormous numbers of typical bacteria in the vascular system. The seeds were carefully preserved, and three months later were planted in the greenhouse. Good germination resulted, and after three months' growth no signs of wilt had occurred in any of the plants.

In July, 1915, a large White Spine cucumber fruit almost full grown was inoculated from a pure culture of the bacterial wilt organism. The fruit became infected and the wilt extended gradually throughout the whole vine to which it was attached. Seeds from this fruit were preserved, and six months later a part of them were planted in the greenhouse. Several plants came up and were under observation for four weeks, but no wilt occurred. A portion of the seeds remaining were used in cultural tests. The seeds were sterilized in the usual way with mercuric-chlorid solution, the seed coats removed under aseptic conditions, and the embryos crushed in sterile bouillon from which plates were poured. No clouding of the bouillon subsequently occurred, and no growth from the plates.

On August 29, 1914, a ripe cantaloupe was collected from a wilted vine near Albany, N. Y. The vascular elements of the cantaloupe con-

tained an abundance of the typical stringy ooze which microscopic examination showed to consist entirely of characteristic bacteria. Seed germination and cultural tests similar to those described for the cucumber gave negative results.

During the latter part of September, 1914, ripe Hubbard squashes were collected from wilted vines at Medina, Malone, and Constable, N. Y. These squashes upon examination showed the same evidence of bacterial wilt as did the cucumbers and cantaloupes referred to above, and in addition a pure-culture isolation of *B. tracheiphilus* was made from the Medina squash, which subsequently gave typical infections when inoculated into healthy cucumber and squash plants. Seed germination and cultural tests from the seeds gave the same negative results as in cucumbers and cantaloupes.

### STOMATAL INFECTIONS

Two inoculation tests with cucumber and one with cantaloupe were made during the summer of 1915, using sterile-water suspensions of the wilt organism (strain R230). The plants were put into tight inoculating cages, and the plants and walls of the cages sprayed with tap water. Two hours later the plants were inoculated by spraying with a very cloudy suspension of bacteria from 7-day-old agar slants. Check plants were inoculated by needle punctures from the same cultures. All the plants were left in the cages tightly closed for 24 hours, and semiopen for two days longer. The punctured checks wilted promptly, but no infection occurred in the uninjured sprayed plants, although they were kept under observation for two months. Another test was made in March, 1916. Three young and three older cucumber plants and four young squash plants were inclosed in a dampened inoculation chamber and sprayed with a tap-water suspension of *B. tracheiphilus* (strain En58 isolated from squash) from a 6-day-old beef-agar slant. Three hours later the plants were again sprayed with this bacterial suspension. This culture was at the same time tested by needle-puncture inoculations into the leaves of two cucumber and two squash plants of the same varieties. The sprayed plants were left in the inoculating chamber in a saturated atmosphere for three days, after which they were held under ordinary greenhouse conditions. After two months no infection had appeared in the sprayed uninjured plants, although the plants inoculated from the same culture by needle punctures all developed typical wilt within one week after inoculation.

A fifth trial was made in April, 1916, using five young and five older cucumber plants. All aerial parts were thoroughly sprayed with a tap-water suspension of the wilt organism from a beef-agar slant 6 days old (strain En57). This culture was tested by needle-puncture inoculations into cucumbers of the same age and variety. The latter inoculations resulted in typical wilt, but the uninjured plants sprayed with the bacterial suspension had shown no signs of infection after five weeks.

In these five direct tests stomatal infection did not occur, thus confirming the observational data during the past two seasons and Dr. Smith's earlier observations and experiments. In hundreds of field and greenhouse observations the stems and leaves of wilted and healthy plants were closely intertwined, exposing in many cases uninjured healthy parts to direct contact with cut and broken infected surfaces. Even here the disease was in no case transmitted.

## DISCUSSION OF OBSERVATIONS

The field observations of the senior writer during the last two seasons, covering the territory from the District of Columbia to eastern Long Island, northward to the Canadian Provinces of Quebec and Ontario, and westward to Michigan, Wisconsin, and Indiana, have abundantly confirmed the experimental evidence outlined above that the striped cucumber beetle and probably also the twelve-spotted cucumber beetle are the principal if not the only carriers of bacterial wilt of cucurbits. It has been suggested that the larvæ of cucumber beetles may also serve as a means of dissemination, but from their habits it would appear that the only possible way in which they could bring about infection is by carrying the organism from the soil into their burrows in the cucumber stems. This appears highly improbable. However, the data at hand do not warrant any definite statement.

Mechanical injuries, such as those resulting from storms, cultivation, etc., and injuries from flea beetles, aphids, and squash bugs have been closely watched in the experimental fields and cages described elsewhere, but no evidence has been obtained of any relation to bacterial wilt.

## WILT CONTROL

The problem of control therefore resolves itself into (1) the finding or developing of cucurbit varieties resistant to bacterial wilt, (2) spraying the plants with a bactericide, or (3) eliminating the beetles through poisons or repellants.

## VARIETY TESTS

Early in the spring of 1915 a preliminary test was made with eight varieties of cucumber planted in pots in one of the department greenhouses. Several plants of each variety were inoculated by needle punctures in the leaves from 6-day-old agar-slant cultures of a single strain of *B. tracheiphilus*. All the inoculated plants contracted the disease and no difference in rapidity of wilting appeared—that is, individual plants of the same variety showed as great differences in rate of wilting as appeared among the different varieties.

In the variety-test block previouslv mentioned (East Marion, Long Island, N. Y.) 32 varieties of cucumber, 39 varieties of cantaloupe, and 25 varieties of squash were planted on June 10, 1915. From 8 to 20 or more hills were given to each variety, 12 being the usual number. Most of the cucumber and squash varieties gave fair to good stands, but the cantaloupes were planted in an exceedingly light sandy soil infested with witch grass, and in consequence of this the seed either did not come

up at all or gave a very poor stand of plants. Only seven of the cantaloupe varieties were in such location and condition as to be included in a summary of results. It was intended at first to inoculate artificially at least one plant of each variety with the wilt organism, in order that all varieties might have an equal chance, but the disease soon became so general over the experimental block that it was thought unnecessary to interfere with its natural spread.

Careful observations were made throughout the season and the number of wilted plants in each variety was noted. Table I gives the percentage of wilted plants for each variety during the season.

TABLE I.—*Percentage of wilt in different varieties of cucumbers, squashes, and cantaloupes at East Marion, Long Island, N. Y., season of 1915*

CUCUMBER

| Variety. | Percentage of wilt. | Variety. | Percentage of wilt. |
|---|---|---|---|
| West India Gherkin | 30 | Improved Long Green | 66 |
| Rollistons Telegraph | 33 | Fordhooks Famous | 70 |
| Emerald | 33 | Vaughans XXX Pickling | 71 |
| Cool and Crisp | 33 | Cumberland Pickling | 75 |
| Vaughans Prolific | 40 | Fordhook Pickling | 77 |
| Lemon | 40 | Early Cyclone | 77 |
| Westfield Chicago Pickling | 44 | Improved White Spine | 77 |
| Snows Fancy Pickling | 45 | Arlington White Spine | 80 |
| Davis Perfect (regular stock) | 50 | Arlington White Spine (U. S. 19300) | 83 |
| Davis Perfect (selected stock) | 50 | Improved Long Green (U. S. 18591) | 83 |
| Noas Forcing | 50 | Early Cluster | 83 |
| Extra Early Long White Spine | 66 | Serpent or Snake | 88 |
| Boston Forcing White Spine | 66 | Carters Model | 90 |
| Improved Jersey Pickling | 66 | New Century | 100 |
| Boston Pickling (U. S. 18589) | 66 | Grand Rapids Forcing | 100 |
| Rockyford Klondyke | 66 | | |
| Japanese Climbing | 66 | | |

SQUASH

| | | | |
|---|---|---|---|
| Mammoth White Bush | 0 | Improved Hubbard | 42 |
| Early White Bush (U. S. 19339) | 0 | Pikes Peak | 42 |
| Vaughans Giant Summer Crookneck | 10 | Delicata | 50 |
| Early White Bush | 12 | Essex Hybrid | 50 |
| Early White Bush (U. S. 19340) | 12 | Delicious | 60 |
| Mammoth Yellow | 14 | Faxons Brazilian | 70 |
| Giant White Summer | 16 | Chicago Market Hubbard | 75 |
| Straight Neck | 20 | Orange Marrow | 87 |
| Bush Fordhook | 26 | Red or Gold Hubbard | 88 |
| Fordhook | 30 | Marblehead | 100 |
| Yellow Bush | 40 | Golden Bronze | 100 |
| Summer Crookneck | 40 | Boston Marrow | 100 |
| | | Vegetable Marrow | 100 |

CANTALOUPE

| | | | |
|---|---|---|---|
| Emerald Green (U. S. 19352) | 9 | Burrell's Gem (U. S. 19312) | 25 |
| Landreths Early Citron | 12 | Burrell's Gem (U. S. 19348) | 25 |
| Rockyford (U. S. 19319) | 15 | Vegetable Peach | 28 |
| Rockyford (regular stock) | 15 | Oval Netted Gem | 66 |
| Netted Gem Rockyford (selected stock) | 18 | | |

None of the 30 varieties of cucumber were free from wilt, the diseased plants in each variety ranging from 30 to 100 per cent. In the 7 varieties of cantaloupe exposed to infection, the wilt ranged from 9 to 66 per cent. Of the 24 varieties of squash, 2 remained free from wilt throughout the season, and in the remaining 22 varieties the disease occurred in 10 to 100 per cent of the plants. Little hope of finding a high degree of resistance is to be noted in the cucumber record. A considerable difference in percentages of wilt is found, but whether this will persist from year to year or is merely accidental can be ascertained only by further trials in different localities and seasons. A greater promise of resistance was evidenced by the squash varieties. In his experiments Dr. Erwin F. Smith, Bureau of Plant Industry, obtained infection in squashes with *B. tracheiphilus* obtained from wilted squash plants, but with cultures obtained from cucumbers squash infections were rare or where they did occur failed to extend beyond the immediate vicinity of inoculation.

Experiments relative to the infection of squash plants by means of cultures of *B. tracheiphilus* obtained from cucumbers, cantaloupes, and squashes are not yet completed. However, up to the present time, 15 strains from cucumber, 1 from cantaloupe, and 7 from squash have been tested by inoculation into these three hosts. All the strains have proved infectious on cucumber and cantaloupe. Of the 15 cucumber strains inoculated into the Yellow Crookneck and Early White Bush squashes, 7 have given no infection, 2 (En66 and En68) have given doubtful signs of incipient wilt, 4 (En68, En109, R305, and R307) primary wilt (not extending beyond the inoculated leaves), and 2 (R308 and En108) wilt involving the entire plant. The single cantaloupe strain in most cases failed to infect squash. In those cases where infection did occur, the signs did not extend far beyond the inoculation punctures. All the squash strains were infectious to squash, varietal differences, however, being evident.

Among the common cultivated cucurbits cucumbers appear to be the most susceptible, and following them in succession should be placed cantaloupes, squashes, and pumpkins, with watermelons (*Citrullus vulgaris*) as most resistant. So far as the writers know, bacterial wilt has been reported but once as occurring naturally upon watermelons, and this case was reported without accompanying proof.[1] The ordinary watermelon wilt is caused by a species of Fusarium.

Summarizing the season's work upon cucurbit varieties, together with the general field observations of the senior writer, it may be stated that there is little hope of controlling the disease in the cucumber through host resistance to the parasite. The cantaloupe and squash, especially

[1] Selby, A. D. Certain troublesome diseases of tomatoes and cucurbits. *In* Ann. Rpt. Columbus Hort. Soc. 1896, p. 113. 1897.

the latter, showed a considerably greater evidence of resistance. For these plants, therefore, this method of control is at least worthy of further investigation, but up to the present time the observations and experiments do not justify definite conclusions.

## SPRAYING

In two fields situated near the variety-test block a series of spraying experiments was carried out in 1915 upon the Fordhook Famous cucumber, planted on June 5 and 7, and Woodruffs Hybrid cucumber, planted on June 1. The relation between the striped cucumber beetle and wilt in these two fields has already been detailed (p. 420 and fig. 1). The relative merits of Bordeaux mixture alone, Bordeaux mixture with lead arsenate, and lead arsenate alone were tried out by spraying different plots with each of these three mixtures on a succession of dates, beginning June 25 and continuing at intervals of 5 to 10 days thereafter (fig. 2).

To determine the best time for treatment, the first application of the Bordeaux-mixture-lead-arsenate combination was made upon different plots at successive dates. The first application was made on June 25, just as the first true leaves had opened on the cucumber plants, and at each succeeding application a new plot was added. In every case a check plot was left between the two successively sprayed plots. In field 2 each plot consisted of three parallel rows, each row 21 feet long. In field 1 the plots were about twice this size. The first three applications of Bordeaux mixture were made with a weak suspension (2:2:50) in order not to injure the young plants, but in the later treatments the strength was gradually increased to the 4:6:50 formula. In all cases where lead arsenate was used it was applied at the rate of 2 pounds to 50 gallons of liquid. No appreciable injury from any of the spray mixtures was observed.

The relative amount of control effected in field 1 at different dates of application is graphically shown in figure 3.

The spray treatments were conducted as follows: Plot 1 (fig. 3a) received its first application of Bordeaux and lead arsenate on June 25, and additional sprayings at intervals of 5 to 10 days throughout the season. In plots 2, 3, and 4 (fig. 3, b, c, and d), the first applications were made on July 6, 14, and 19, respectively, and further sprayings were made at intervals of 5 to 10 days as in plot 1. It will be noted that most of the infections had occurred before the third treatment, July 14, for in plot 3 and its corresponding check the number of wilted plants was about the same. In the first two plots there was much less wilt than in the corresponding unsprayed plots, the first sprayed plot showing by far the best results. There would be a still greater difference between plot 1 and its untreated check were it not for the facts that the latter was only about three-fourths the size of the sprayed plot and that it received one applica-

tion of a dust insecticide to prevent the total destruction of the plants by striped cucumber beetles.

The relative amount of control given by the three kinds of spray mixture tested is shown in figure 2, in which the number of wilted plants in each sprayed and check plot is given. It will be noted that the lead arse-

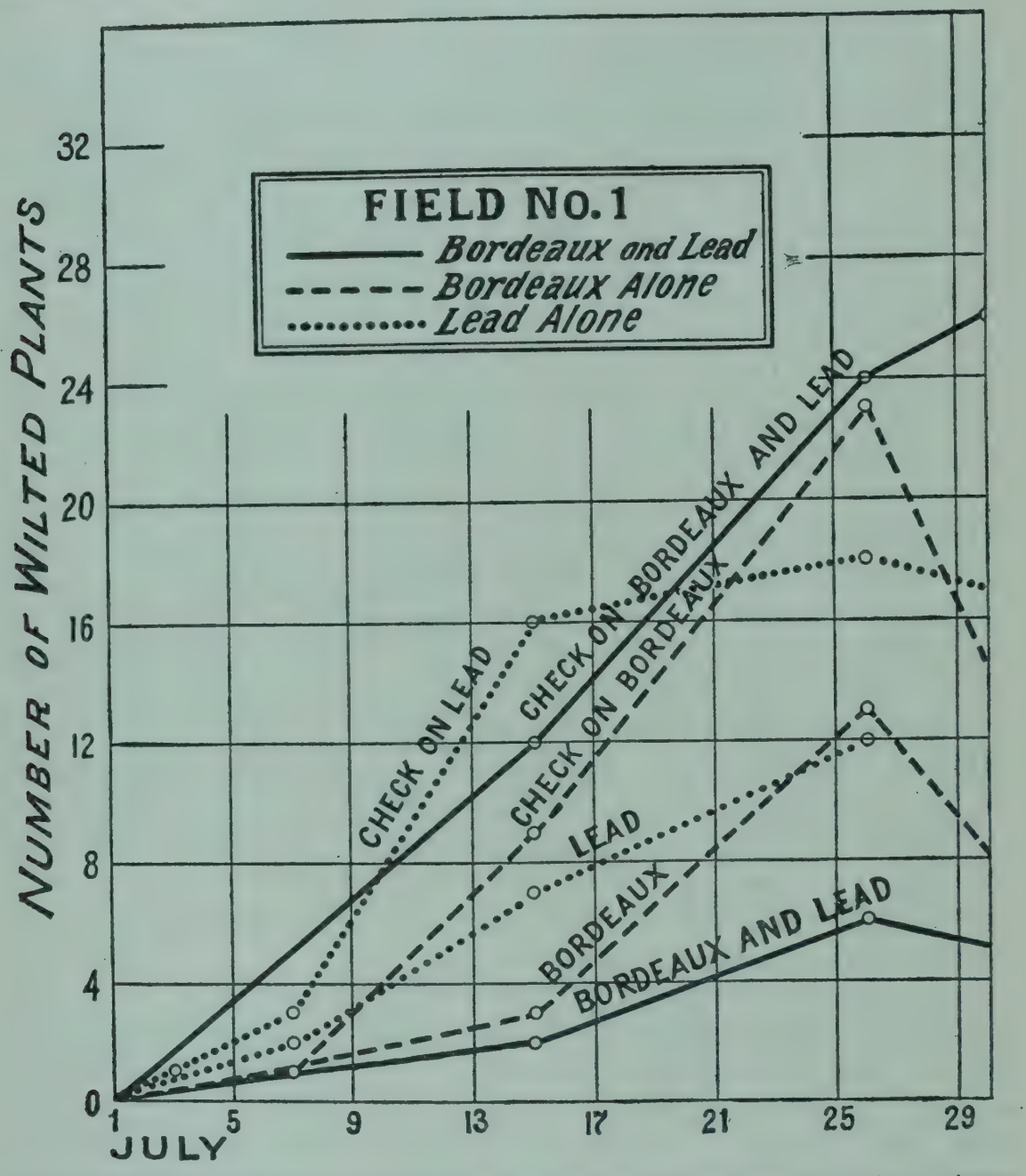


FIG. 2.—Comparison of relative wilt control of Bordeaux mixture plus lead arsenate, Bordeaux mixture alone, and lead arsenate alone in field 1, East Marion, Long Island, season of 1915.

nate and Bordeaux mixture combined gave better results than either used alone.

The results obtained in field 2 are corroborative of the data graphically shown for field 1 (fig. 2 and 3), but the control effected was not quite so striking, since the plants were nearly a week older than those in field 1 at the time of the first spraying. Furthermore, the stand was poor in some

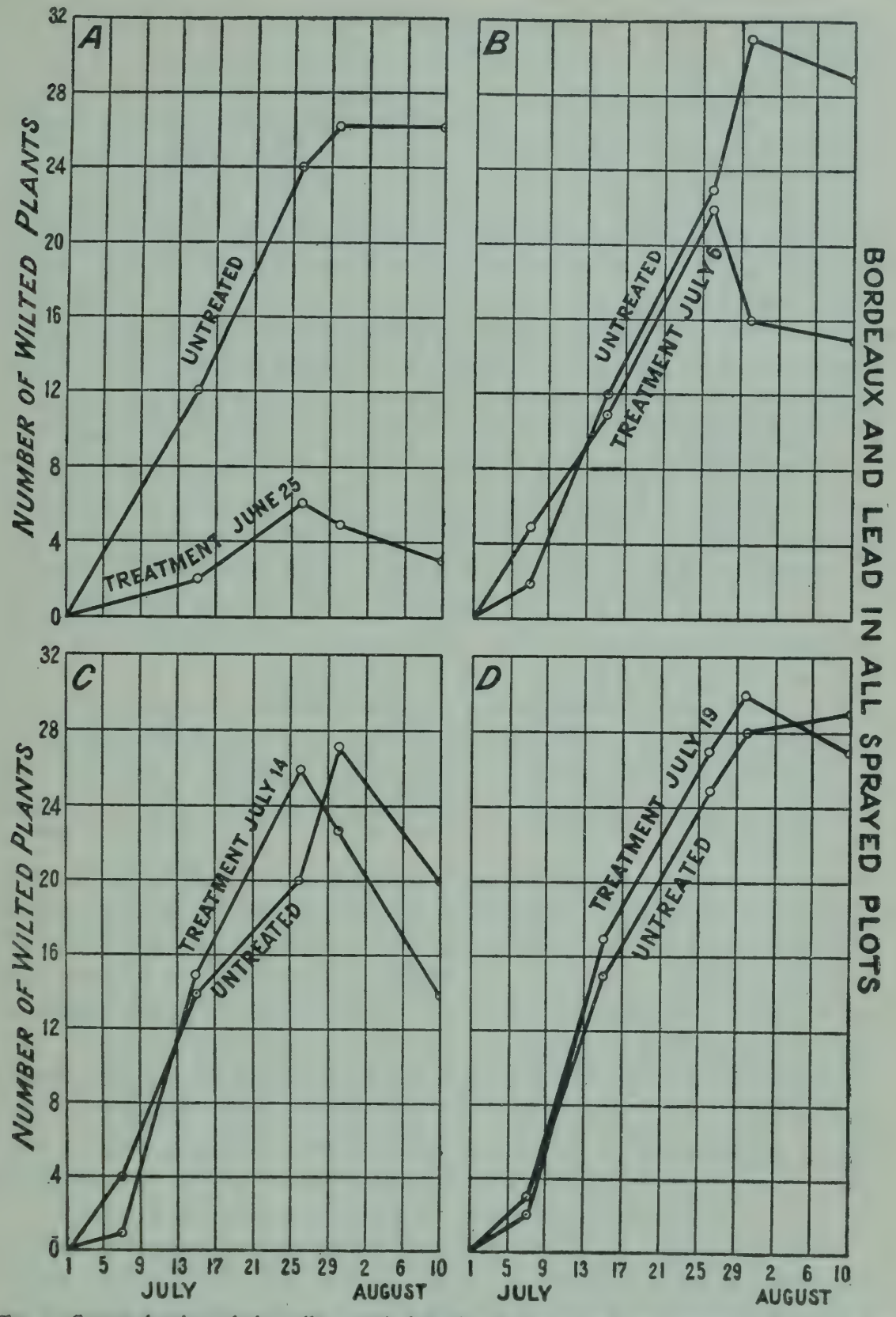


FIG. 3.—Curves showing relative wilt control of Bordeaux mixture and lead arsenate with date of first application as a variant in field 1, East Marion, Long Island, season of 1915.

parts of the field and the beetles appeared here a few days earlier than in field 1.

Field observations indicate that the results obtained were due to a bactericidal and repellent action of the Bordeaux mixture and lead-arsenate combination, and probably in part to an insecticidal action by the latter ingredient. The beetles were less frequent on the sprayed than on the unsprayed plots, and among the sprayed plants injured by beetles there was apparently a smaller percentage of infection resulting than among similar unsprayed plants. That is, the control effected by the Bordeaux mixture alone was apparently due to its repellent and bactericidal action, and that by the lead arsenate alone to its repellent and insecticidal action, while the more complete control by the two mixtures together was due to a combination of their bactericidal, repellent, and insecticidal properties.

The bactericidal action of Bordeaux mixture has been further investigated in a series of six greenhouse tests, in which sprayed and unsprayed leaves of potted plants were inoculated in as nearly an identical manner as possible by needle punctures from cultures of the same strain of organism. The spray used was 2:3:50 Bordeaux, and this was allowed to dry thoroughly on the leaves before inoculating. In most cases the plants were not inoculated until 24 hours after spraying.

In the first test, December 2, 1915, three weeks after planting, seven unsprayed and seven sprayed Chicago Pickling cucumber plants were inoculated from 1-week-old beef-agar slant cultures. After 15 days the unsprayed plants showed 100 per cent of infection, and the sprayed plants 29 per cent.

In the same way and at the same time a test was carried out on three varieties of cantaloupe—Rockyford, Sweet Air, and Baltimore Nutmeg. Thirty-five inoculations were made into unsprayed plants and 37 into sprayed plants. There was no apparent difference in susceptibility among the three varieties used. Of these inoculations the unsprayed gave 95 per cent of infection and the sprayed leaves 46 per cent.

In a third test (Jan. 8, 1916), Chicago Pickling cucumbers planted November 13, 1915, were used. In this test 36 unsprayed and 37 sprayed plants were inoculated with the wilt organism, using agar slants 9 days old. After 19 days it was found that 92 per cent of the unsprayed and 35 per cent of the sprayed cucumbers had contracted the disease.

A further trial was made (Jan. 19, 1916) with 19 Chicago Pickling cucumbers planted October 29, 1915. In the case of these older cucumbers unsprayed and sprayed leaves on the same plant and as nearly of the same age and appearance as possible were used for inoculation. Both sprayed and unsprayed leaves had been dusted with flowers of sulphur for the control of powdery mildew, and this treatment, together with the age of the plants, considerably reduced the infection. However, even here the unsprayed leaves gave 63 per cent and the sprayed leaves 11 per cent of infection.

Two more tests (Jan. 19, 1916) were made with Baltimore Nutmeg cantaloupes planted November 13, 1915, using a bacterial strain of low virulence (strain R311) and one of high virulence (strain R304). The cultures of these two strains used for inoculation were beef-agar slants 10 days old. With the former strain 10 unsprayed and 9 sprayed plants were inoculated, and these gave, respectively, 40 and 11 per cent of infection. With the highly virulent strain 16 unsprayed and 17 sprayed plants were inoculated. These gave, respectively, 94 and 24 per cent infection.

Remarks: It will be seen that in all cases the presence of Bordeaux mixture on the leaves greatly reduced infection, and an average of the six trials gives 80.6 per cent of infection in the unsprayed against 26 per cent of infection in the sprayed plants. These results can scarcely be considered as accidental, and they strongly confirm field observations regarding the bactericidal effect of Bordeaux mixture. Furthermore, the natural mode of inoculation is considered identical with the method used in these tests, for in the one case the organism is pricked into the leaf tissues by the mouth parts of the cucumber beetle and in the other case by the inoculating needle.

### Wet and Dry Inoculations

On January 8, 1915, an experiment was conducted to determine the effect of wet and dry inoculations into sprayed and unsprayed plants. In this test 68 cucumber plants were used. The inoculations were all made in a uniform manner by needle punctures into the two youngest, fully opened leaves of each plant. Of these plants 34 were sprayed with Bordeaux mixture and 17 were inoculated before the Bordeaux mixture had dried. The remaining 17 were inoculated about 2 hours later when the Bordeaux mixture was thoroughly dry. At the same time 34 unsprayed plants were inoculated, 17 while dry and 17 immediately after sprinkling with tap water. All of the plants were shaded from the sun until the following day. At the end of 19 days after inoculation 95 per cent of the unsprayed plants inoculated when wet had contracted the wilt and 88 per cent of those inoculated when dry. In the sprayed plants there was 33 per cent of infection among those inoculated before drying and 36 per cent among those inoculated after drying.

As will be seen, the percentage relations between infection in wet and dry leaves vary inversely in the sprayed and unsprayed plants. The difference is small, but it occurs in the direction to be expected from known facts concerning conditions favorable to infection. In the absence of bactericidal substances a moist leaf surface presents a better environment for infection by the bacteria; but when a bactericide is present which is effective in solution the maximum effect occurs in the presence of water. This is exactly the result obtained in the experiment under discussion.

SUMMARY

(1) In fields where wilt had largely destroyed the cucumber crop during the preceding season the disease did not appear in 1915 on cucumbers in 48 beetle-proof cages. On the other hand, wilt was very prevalent in those fields on all sides of the cages. In a large number of greenhouse tests where one out of two plants in a pot was inoculated and wilted to the ground the second plant in no case contracted the wilt. The inoculations by means of bacterial suspensions poured on the soil around potted cucumber plants showed a small but varying percentage of wilt. Root injuries were found in most of these cases of root infection. Apparently infection does not enter the uninjured root system from the soil.

(2) In all cases seeds from diseased fruits failed to produce diseased plants, and cultures from such seeds in no case gave the wilt organism, but further tests should be made.

(3) In the tests made stomatal infection did not occur.

(4) The experiments thus far completed show that cucumber beetles (*Diabrotica* spp.) are the most important, if not the only, summer carriers of the wilt organism (*Bacillus tracheiphilus*) and that at least one species (*D. vittata*) is capable of carrying the wilt over winter and infecting the spring planting of cucumbers. In the tests by the writers the squash bug (*Anasa tristis*), the flea beetle (*Crepidodera cucumeris*), the melon aphis (*Aphis gossypii*), and the twelve-spotted lady-beetle (*Epilachna borealis*) have failed to transmit the disease.

(5) In the field experiments during one season with many different varieties of cucurbits, the greatest difference in resistance was shown by varieties of squash, in which the percentage of infection varied from 0 to 100. The varieties of cucumber and cantaloupe, while showing some difference in their susceptibility to the wilt, give much less promise of control by varietal resistance.

(6) In the spraying experiments of 1915 wilt was effectively controlled by early treatments with a combination of Bordeaux mixture and arsenate of lead. Plots sprayed with either mixture alone showed much less wilt than unsprayed plots, but control was not as complete as where the two were used together. Both field observations and greenhouse experiments indicate that the wilt control is effected through the bactericidal action of Bordeaux mixture, the insecticidal action of arsenate of lead, and the repellent action of both against the cucumber beetles.

(7) Inasmuch as it has been definitely proven that the striped cucumber beetle (*D. vittata*), and also the twelve-spotted cucumber beetle (*D. duodecimpunctata*), are the most active carriers of the bacterial wilt, it becomes necessary to control the insects in order to prevent the disease. This phase of the work will be actively undertaken in cooperation with the Bureau of Entomology during the coming season.

## PLATE LIII

Two wilted cucumber plants which contracted bacterial wilt at beetle gnawings of the leaves marked *x*. Three healthy, uninjured plants in same hill are also shown. From field 1, East Marion, Long Island, N. Y., July 19, 1915.

X
X

1

2

3

4

## PLATE LIV

Plots in field 1, East Marion, Long Island, N. Y., 1915. The poor stand in figures 2 and 3 was due entirely to bacterial wilt.

Fig. 1.—Plot sprayed with Bordeaux mixture and lead arsenate, beginning June 25. Photographed September 20, 1915.

Fig. 2.—Plot sprayed with Bordeaux mixture and lead arsenate, beginning July 19, after most of the striped-beetle injury had occurred. Photographed September 20, 1915.

Fig. 3.—Plot sprayed with Bordeaux mixture and lead arsenate, beginning July 27. Practically no beetle-injury occurred after this date. Photographed September 20, 1915.

Fig. 4.—General view of field, showing cages and meteorological-instrument shelter. Photographed July 10, 1915.

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# CORRELATED CHARACTERS IN MAIZE BREEDING

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## INTRODUCTION

The study of correlations as an aid to plant breeding was at one time thought to be full of promise, but in recent years little use has been made of correlations by practical workers. From this fact it might appear that the early hopes were unwarranted, and that correlation is a factor of little or no importance. It must be conceded that the elaborate calculations of correlation coefficients have in few instances proved of value to the practical breeder, yet it must be admitted on reflection that nearly all successful breeding has in reality been made possible by the fact that correlations exist.

In plant breeding the improvement and preservation of varieties has largely resulted from the ability of the breeder to recognize desirable types, and the existence of definite types is in itself a manifestation of the correlation of characters. The existence of types must mean that there are many individuals that present approximately the same combination of characters, and this is exactly what correlation implies. The characteristics of the desired type are recognized by the breeder even though they may not be formulated, and varieties are seldom established by selection confined to a single character. If the study of correlations has appeared to have little bearing on plant breeding, it must be that we have been studying the wrong characters or studying them in the wrong way.

In the improvement of maize varieties (*Zea mays*), as with other plants, the recognition of types has been an important factor. The selection, however, has been almost entirely confined to the ear. In a field of any commercial variety it is easy to recognize differences in the plants, but even after long familiarity with the variety the plants refuse to be classified into distinct groups. This difficulty in recognizing types among maize plants greatly increases the difficulty of breeding this crop.

The lack of recognizable types in maize is very different from the condition that obtains, for example, in cotton (*Gossypium* spp). With cotton,

skilled breeders are able to detect deviation from type even in the early stages of development and the practice of roguing can proceed with certainty. It appears that when a cotton plant deviates from type it deviates in a more or less definite way and in many particulars, or, in other words, there are a number of coherent or correlated characters.

It seemed desirable to determine whether the difficulty in recognizing types in maize is due to a lack of familiarity with the plants or whether there is a fundamental difference between the heredity of maize and that, for example, of cotton.

In the seed characters of maize a definite correlation has been found between the color of the aleurone and the texture of the endosperm (Collins and Kempton, 1913). Correlations have also been noted between the color of the silk and the color of the anthers (Webber, 1906), and between the color of the seed and the color of the cob, dwarfness and broad leaves, and between stamens in the ear and club-shaped tassels (Emerson, 1911). There was, therefore, abundant reason for suspecting that the difficulty of recognizing types among maize plants might be due to a lack of sufficient discrimination, and it was with the idea that correlations were the rule rather than the exception that the present experiment was undertaken. Contrary to expectation, the results give evidence that for the varieties and characters studied there is almost a complete absence of genetic correlations.

## CLASSIFICATION OF CORRELATIONS

Correlations may be classified in a great variety of ways and with almost any degree of refinement. As with any classification of organic activities, no particular grouping can be made to serve all purposes, for it is necessary to divide the subject in different planes.

For purposes of the present discussion correlations, or the mutual relations of characters, are divided into three main groups, to which the names "physical," "physiological," and "genetic" may be applied.

PHYSICAL CORRELATIONS are those in which the relation is obviously causal. In many instances correlations of this kind are little more than different names for the same phenomenon, or parts of the same phenomenon, as when increased weight is correlated with increased height. In physical language one of the characters would be described as a function of the other.

PHYSIOLOGICAL CORRELATIONS are those where both characters are the result of the same physiological tendency, as when long internodes in the main stalk are correlated with long internodes in the branches. This may be looked upon as a general tendency to elongated growth that is manifested in different parts of the plant.

GENETIC CORRELATIONS cover the large residue of correlations, the nature and causes of which are questions of controversy, but which are

associated with the method or mechanism of heredity. An example of this type of correlation is shown in the association of yellow petals and deeply lobed leaves in Egyptian × Upland cotton hybrids.

This classification differs from those proposed by Webber (1906) and East (1908) chiefly in placing physical correlations outside the pale of biological correlations. Most of those correlations classed by Webber as morphological would here be considered as physical. This distinction is made because it seems to the writer that the relation between length and weight, for example, is inherent in the properties of matter and is not a biological phenomenon. Certainly a relation of this kind would be found in stones or any inanimate objects selected at random.

Since physiological functions are always directly or indirectly induced by or at least associated with environmental stimuli, Webber's environmental and physiological correlations are here combined. That the examples of physiological correlations cited by Webber are reverse or negative correlations need not confuse the issue, since by simply stating the relation in other terms the correlations can be made to appear as positive.

The distinction between physiological and genetic correlations may not always be easy to apply, and the apparent need of it may disappear entirely with a more complete knowledge of inheritance and methods of growth. For the present, however, the distinction will be useful even if physiological correlations are confined to pure lines or asexually propagated stocks where differences in inheritance can be eliminated. To ascribe the long internodes of the main stem and branches to the activity of a single determiner or gene is hardly less futile than to offer the same explanation for the correlation between the length and weight of inanimate objects. If the one is inherent in the properties of matter, the other is inherent in the properties of plants.

All examples of genetic correlation are exceptions to the third law of Mendel, which implies that characters are redistributed in the perjugate generations of a hybrid in accordance with the laws of chance. Conversely, all instances in which Mendelian ratios, other than the 3 to 1 ratio of a monohybrid, are followed with exactness demonstrate the action of this third law and the absence of correlations among the factors which make up the characters. It should be kept in mind, however, that multiple hybrid ratios have seldom been determined with any great degree of accuracy, so that correlations, unless of a pronounced type, would escape detection.

The significant factor in genetic correlations is the grouping of the characters in the ancestry and not the inherent properties of the characters themselves. Thus, when colored aleurone and horny endosperm are found to be correlated in the progeny of a hybrid, involving colored and white aleurone and horny and waxy endosperm, it does not indicate

any attraction between colored aleurone and horny endosperm, but rather that one of the parents had colored aleurone and horny endosperm, while the other parent had white aleurone and waxy endosperm. This tendency for parental combinations to reappear has been called "coherence," and, so far as known, all genetic correlations thus far recorded are of this nature.

Many investigations have been devoted to correlations in agricultural plants, but unless the special class of correlations covered by coherence is kept in mind the results are likely to be disappointing to the breeder. Cylindrical ears of maize may be correlated with high yield in one population and the opposite result be reached in another case, depending on whether these characters were introduced into the population under investigation from the same parent or from different parents.

There are doubtless many physiological correlations that may be detected by elaborate measurements, but unless the observations are confined to asexually propagated groups or to those of which the ancestry has been carefully studied, there will always remain the uncertainty whether there is an inherent physiological relation between the development of the two characters or whether the correlation is the result of ancestral combinations. The distinction is not without practical importance, for a physiological correlation can not be reversed by any direct means at the disposal of the breeder—that is, without evoking mutation or some form of evolutionary change—while, if the correlation is genetic, the relation between the characters may usually be reversed by a few generations of selection in the desired direction.

Two principal theories have been advanced to explain genetic correlations. These are the theory of reduplication (Bateson, Saunders, and Punnett, 1906) and the theory of linkage developed by Morgan and his students (1915) from studies of the fruit fly *Drosophila ampelophila*. Both of these theories deal with characters which are alternative, both having been derived from the study of Mendelian inheritance.

With the idea that continuous inheritance is to be looked upon as a complicated form of alternative inheritance, it should be interesting to learn what light the study of genetic correlations between characters that are blended in inheritance may throw on the theories of reduplication and linkage. The experiments described below constitute a preliminary attempt to extend the study of genetic correlations to characters that are continuously inherited.

## METHODS OF DISTINGUISHING BETWEEN PHYSIOLOGICAL AND GENERIC CORRELATIONS

To determine with certainty that a given correlation is physiological and not genetic, it would be necessary to demonstrate the existence of the correlation in material where all the individuals possessed the same

hereditary tendencies with respect to the characters studied. Theoretically this is only possible in asexually propagated groups. Approximately pure lines may be obtained where self-pollination is possible, so that if correlations are found they may with assurance be considered physiological. In maize, however, even to approximate pure lines produces such abnormal conditions that some other method of study must be sought.

Even in maize it would seem that the question might be approached by comparing the degree of correlation in types or varieties having a relatively restricted ancestry with that observed in the perjugate generations of hybrids between two contrasting forms.

An equally satisfactory method is to compare the degree of correlation in the first or conjugate generation of a hybrid with that of the perjugate generations. Where the conjugate generation is all descended from a single cross, the gametic differences should be no greater than self-pollinated progenies of the parents.

Unfortunately in our experiment the number of first-generation individuals was too limited to detect any but relatively large correlations. Wherever data were available, additional evidence has been presented from the behavior of the original varieties. Although a large number of plants of both parent varieties have been grown and measured, the data have been secured in different localities and in different years, a fact that renders many of the measurements unavailable for these studies.

## DESCRIPTION OF MATERIAL

The hybrid that afforded the data for the present paper was a cross between Waxy Chinese and Esperanza, two varieties of maize separated by a number of definitely contrasted characters. The hybrid was made at Lanham, Md., in 1908.

The peculiarities of the Waxy Chinese variety (Pl. LV–LVI) have been described elsewhere (Collins, 1909).

The particular plant used as female parent of the hybrid was grown from the original seed imported from China. The individual notes taken in 1908 give the following details:

> Height, 167 cm. Length of fifth leaf from the top, 83 cm. Width of fifth leaf, 9 cm. Leaf sheath smooth. Nodes above the ear, 4. Suckers, 0. Plant rather open, but distinctly one-sided.

The Esperanza variety belongs to a peculiar type of maize that appears to be confined to the table-lands of Mexico, the *Zea hirta* of Bonafous (1829). This variety was obtained in 1906 from Esperanza, Pueblo, Mexico, by Mr. H. Pittier, of the Bureau of Plant Industry (Pl. LVIII and LIX).

The plant that was the male parent of the hybrid was raised from seed grown at Lanham, Md., in 1907. Regarding the 1907 plants, the notes

state that all were typical of the hairy Mexican type, ranging from 150 to 210 cm. in height. The notes recorded for the 1908 plant used in making the hybrid state that it was typical of the variety except for a general shortening of the internodes. It was 105 cm. high, had three tassel branches, four nodes above the ear, and the fifth leaf from the top measured 83 by 14 cm.

Sixteen first-generation plants were grown in 1909. Three pure-seed ears that provided seed for the second generation are designated as No. 1, 2, and 3. Four plants entered into the parentage of these three ears. No. 1 and 2 were reciprocals. No. 1 resulted from pollinating plant 225 by plant 226. No. 2 by pollinating plant 226 by plant 225. No. 3 was the result of pollinating plant 262 by plant 263. The ears on all three of the first-generation plants that produced ears 1, 2, and 3 showed the usual mixture of waxy and horny seeds that result from crossing the Waxy Chinese and a corneous or horny variety. The notes taken on the four first-generation plants are presented in Table I.

TABLE I.—*Description of four first-generation maize plants grown in 1909*

| Plant No. | 225 | 226 | 262 | 263 |
|---|---|---|---|---|
| Height.....cm.. | 222 | 228 | 212 | 230 |
| Number of tassel branches..... | 20 | ..... | 18 | 17 |
| Nodes above the ear..... | 5 | 5 | 5 | 5 |
| Length of fifth leaf.....cm.. | 14 | 13 | 14 | 13.5 |
| Width of fifth leaf.....mm.. | 63 | 76 | 86 | 88 |
| Exsert of tassel..... | ..... | 0 | 0 | (a) |
| Arrangement of leaves..... | (b) | (b) | (b) | (c) |

a Exserted. b Scorpioid. c Neither monostichous nor scorpioid.

The final planting was made in 1914. The remnant of seed from the original hybrid ear was planted and furnished 31 first-generation plants. Six rows of approximately 30 plants each were secured of second-generation plants, one row from waxy, and one from the horny seeds of each of the first-generation ears.

The means of the characters measured are given in Table II, and the coefficients of variation in Table III.

TABLE II.—*Mean of different characters in first- and second-generation maize plants*

| Character. | First generation. | Second generation. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ear 1. | | Ear 2. | | Ear 3. | |
| | | Plants from waxy seed. | Plants from horny seed. | Plants from waxy seed. | Plants from horny seed. | Plants from waxy seed. | Plants from horny seed. |
| Height ..... cm. | 223.0 ±2.26 | 195.0 ±4.12 | 194.0 ±3.32 | 192.0 ±3.83 | 190.0 ±4.70 | 182.0 ±4.59 | 185.0 ±4.75 |
| Length of branching space ..... cm. | 15.0 ± .37 | 13.9 ± .37 | 13.4 ± .33 | 13.6 ± .29 | 13.2 ± .32 | 16.5 ± .58 | 13.8 ± .42 |
| Length of central spike ..... cm. | 29.5 ± .54 | 26.8 ± .76 | 27.4 ± .57 | 25.8 ± .49 | 25.4 ± .58 | 24.1 ± .46 | 24.8 ± .37 |
| Number of branches ..... | 28.1 ± .94 | 37.1 ±1.31 | 35.1 ±1.14 | 35.0 ± .84 | 35.4 ±1.02 | 31.7 ±1.14 | 28.9 ±1.27 |
| Number of secondaries ..... | 5.3 ± .31 | 6.9 ± .37 | 5.9 ± .32 | 5.3 ± .32 | 6.3 ± .35 | 3.6 ± .37 | 3.6 ± .41 |
| Number of nodes above ear ..... | 4.8 ± .88 | 4.7 ± .11 | 4.6 ± .10 | 4.7 ± .06 | 4.2 ± .08 | 4.2 ± .06 | 4.6 ± .07 |
| Length of longest leaf ..... cm. | 114.0 ± .64 | 109.0 ±1.16 | 107.0 ±1.09 | 108.0 ± .99 | 107.0 ±1.33 | 99.0 ±1.47 | 104.0 ±1.40 |
| Width of longest leaf ..... cm. | 12.7 ± .16 | 11.9 ± .17 | 11.9 ± .22 | 12.0 ± .17 | 12.5 ± .133 | 10.9 ± .297 | 11.4 ± .220 |
| Ratio of length to width ..... | 9.05± .108 | 9.24± .134 | 9.15± .174 | 9.04± .127 | 8.59± .118 | 9.25± .108 | 9.16± .182 |
| Number of nodes above longest leaf ..... | 8.6 ± .13 | 8.7 ± .21 | 8.1 ± .18 | 8.8 ± .17 | 8.1 ± .23 | 7.3 ± .21 | 8.1 ± .17 |
| Total number of nodes ..... | 24.1 ± .20 | 24.8 ± .40 | ..... | 24.5 ± .24 | 23.7 ± .32 | 22.4 ± .31 | 22.2 ± .20 |
| Number of sheaths with hairs ..... | 9.5 ± .17 | 8.4 ± .29 | 8.8 ± .22 | 7.8 ± .32 | 8.1 ± .28 | 6.4 ± .22 | 7.5 ± .22 |
| Number of sheaths encircled by hairs ..... | 2.9 ± .36 | 1.6 ± .37 | 3.1 ± .32 | 1.4 ± .32 | 2.4 ± .41 | .2 ± .08 | 1.0 ± .26 |
| Length of hairs ..... mm. | 4.9 ± .14 | 4.9 ± .13 | 4.7 ± .10 | 4.8 ± .12 | 4.8 ± .64 | 4.5 ± .13 | 4.7 ± .16 |
| Density of spikelets ..... | 34.0 ±1.31 | 31.2 ± .85 | 32.6 ±1.46 | 32.6 ±1.33 | 31.9 ± .82 | 35.3 ±1.19 | 39.5 ±1.52 |
| Length of glumes ..... mm. | 9.3 ± .12 | 9.6 ± .12 | 10.1 ± .08 | 9.8 ± .14 | 10.2 ± .14 | 9.0 ± .14 | 8.5 ± .20 |
| Number of erect blades ..... | 2.7 ± .23 | 2.4 ± .24 | 2.5 ± .25 | 3.1 ± .34 | 2.6 ± .32 | 2.4 ± .22 | 2.7 ± .24 |
| Angle of tassel axis ..... (°) | 44.3 ±3.78 | 46.0 ±6.30 | 42.0 ±3.49 | 37.0 ±3.80 | 42.5 ±4.93 | 45.3 ±5.62 | 36.2 ±5.67 |
| One-sidedness ..... | 5.4 ± .38 | 4.1 ± .28 | 3.9 ± .33 | 4.9 ± .37 | 4.2 ± .30 | 4.5 ± .31 | 4.0 ± .3 |
| Number of rows, upper ear ..... | 18.3 ± .36 | 18.5 ± .64 | 19.6 ± .48 | 19.0 ± .41 | 18.3 ± .36 | 17.1 ± .35 | 17.9 ± .48 |
| Number of rows, second ear ..... | 17.2 ± .52 | 19.8 ± .73 | 18.2 ± .86 | 17.8 ± .56 | 17.4 ± .51 | 15.1 ± .50 | 16.7 ± .46 |
| Number of husks, upper ear ..... | 12.9 ± .25 | 11.1 ± .40 | 11.2 ± .31 | 11.0 ± .32 | 11.3 ± .33 | 11.8 ± .30 | 11.6 ± .41 |
| Number of husks, second ear ..... | 12.7 ± .35 | 10.3 ± .54 | 11.3 ± .37 | 9.7 ± .25 | 11.7 ± .38 | 11.9 ± .42 | 11.8 ± .41 |
| Exsert of tassel ..... cm. | − 3.05 ± .58 | − 2.77± .41 | − 3.17± .40 | − 3.05± .33 | − 2.68± .45 | − .353± .87 | − .063 ±1.02 |

TABLE III.—*Coefficient of variation of characters in first- and second-generation maize plants*

| Character. | First generation. | Second generation. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ear 1. | | Ear 2. | | Ear 3. | |
| | | Plants from waxy seed. | Plants from horny seed. | Plants from waxy seed. | Plants from horny seed. | Plants from waxy seed. | Plants from horny seed. |
| Height.....cm.. | 8.0± 0.7 | 14.7± 1.5 | 13.0± 1.1 | 14.5± 1.4 | 19.0± 1.8 | 14.9± 1.8 | 17.0± 1.8 |
| Length of branching space.....cm.. | 19.3± 1.8 | 20.4± 1.9 | 19.6± 1.8 | 15.8± 1.5 | 18.3± 1.7 | 22.0± 2.5 | 20.6± 2.2 |
| Length of central spike, cm..... | 14.5± 1.3 | 19.7± 2.0 | 15.2± 1.5 | 13.8± 1.4 | 17.5± 1.6 | 11.6± 1.4 | 9.7± 1.0 |
| Number of branches..... | 26.3± 2.5 | 27.0± 2.7 | 25.2± 2.4 | 17.5± 1.7 | 22.0± 2.1 | 22.0± 2.7 | 29.1± 3.3 |
| Number of secondaries.. | 46.4± 4.9 | 40.4± 4.2 | 42.4± 4.5 | 43.6± 4.7 | 41.9± 4.6 | 62.0± 9.1 | 76.2±11.6 |
| Number of nodes above ear..... | 14.4± 1.3 | 17.4± 1.7 | 17.3± 1.5 | 9.6± 1.0 | 14.3± 1.4 | 8.3± .9 | 10.4± 1.1 |
| Length of longest leaf, cm..... | 4.4± .4 | 8.1± .7 | 8.2± .7 | 6.7± .8 | 9.7± 1.0 | 8.7± .9 | 9.4± 1.0 |
| Width of longest leaf, cm..... | 10.0± 2.5 | 10.7± 1.0 | 15.1± 1.3 | 10.4± 1.0 | 8.2± .7 | 12.8± 1.3 | 17.4± 1.9 |
| Ratio of length to width. | 9.3± .8 | 11.0± 1.0 | 10.7± 1.0 | 10.2± 1.0 | 10.5± .9 | 12.9± 1.4 | 11.7± 1.3 |
| Number of nodes above longest leaf..... | 12.1± 1.1 | 18.4± 1.7 | 17.5± 1.6 | 14.2± 1.4 | 21.8± 1.9 | 18.8± 2.0 | 13.1± 1.4 |
| Total number of nodes.. | 6.2± .6 | 10.9± 1.1 | ..... | 6.8± .7 | 9.1± .9 | 8.0± 1.0 | 5.7± .7 |
| Number of sheaths with hairs..... | 14.1± 1.2 | 26.2± 2.5 | 19.8± 1.8 | 29.4± 3.2 | 26.4± 2.5 | 23.3± 2.5 | 19.1± 2.1 |
| Number of sheaths encircled by hairs..... | 100.0±15.0 | 170.0±42.0 | 83.4±11.1 | 169.0±42.0 | 132.0±25.0 | 225.0±78.2 | 175.0±50.0 |
| Length of hairs....mm.. | 22.4± 2.0 | 19.7± 1.9 | 17.2± 1.5 | 18.5± 1.9 | 10.4± 1.0 | 18.6± 2.3 | 21.3± 2.3 |
| Density of spikelets..... | 30.3± 2.9 | 20.2± 1.9 | 36.2± 3.5 | 29.7± 3.1 | 19.8± 1.9 | 21.6± 2.4 | 24.8± 2.8 |
| Length of glumes..mm.. | 10.7± 1.0 | 9.1± .9 | 6.8± .6 | 10.2± 1.0 | 10.5± 1.0 | 9.1± 1.0 | 15.5± 1.7 |
| Number of erect blades.. | 57.4± 6.9 | 68.7± 9.8 | 66.9± 9.5 | 60.6±10.1 | 75.0± 9.8 | 54.2± 8.0 | 51.3± 7.5 |
| Angle of tassel axis...(°).. | 67.2± 8.9 | 98.2±16.5 | 66.7± 7.9 | 74.4±10.4 | 89.5±13.1 | 77.9±12.8 | 101.0±18.6 |
| One-sidedness..... | 53.6± 6.4 | 48.8± 5.7 | 56.5± 7.5 | 49.6± 6.6 | 52.6± 6.4 | 46.4± 5.9 | 47.8± 6.3 |
| Number of rows, upper ear..... | 13.7± 1.4 | 20.7± 2.5 | 17.2± 1.7 | 13.8± 1.8 | 13.7± 1.4 | 12.7± 1.4 | 16.3± 1.9 |
| Number of rows, lower ear..... | 18.6± 2.0 | 15.5± 2.5 | 19.9± 3.3 | 12.4± 1.6 | 15.0± 2.0 | 13.8± 1.7 | 13.0± 1.9 |
| Number of husks, upper ear..... | 15.1± 1.4 | 24.6± 2.6 | 20.6± 2.0 | 18.8± 2.0 | 20.2± 2.1 | 15.3± 1.8 | 22.7± 2.6 |
| Number of husks, lower ear..... | 19.3± 2.0 | 23.1± 3.6 | 14.4± 2.2 | 11.6± 1.8 | 19.5± 2.4 | 15.8± 2.5 | 18.0± 2.5 |

A comparison of these tables shows that the first-generation plants exceeded the second-generation plants in height, length of branching space, length of central spike, length and width of longest leaf, number of nodes above the longest leaf, number of leaf sheaths with hairs, and number of single-ranked blades. The second-generation plants exceeded the first-generation plants in the number of tassel branches. In the other characters there was no significant difference between the means of the first and second generation plants.

The first-generation plants were distinctly less variable than the second-generation plants in height, length, and width of longest leaf, number of nodes above the longest leaf, total number of nodes, and number of leaf sheaths with hairs. The first-generation plants were more variable with respect to the length of the tuberculate hairs and density of spikelets.

The least variable character measured was the length of the longest leaf. The total number of nodes was also comparatively uniform. The very high coefficient of variation for the number of sheaths encircled by hairs results in part from the alternative nature of this character.

In the progeny of the reciprocal ears 1 and 2 there are no really significant differences. The progeny of ear 3, however, which descended from entirely different first-generation plants, shows a number of differences from the remainder of the second-generation plants.

Although the average height of the plants from all these ears is practically the same, the progeny of ear 3 shows smaller values for a number of other dimensional characters. The number of branches, primary and secondary, length of leaf, total nodes, length of glumes, and number of rows of grains are all slightly lower. With the exception of length of leaf and length of glumes, these differences might be interpreted as indicating a more pronounced development of the Esperanza characters. The same may be said of the exsert, which is higher in ear 3. In the development of tuberculate hairs, on the other hand, the progeny of ear 3 was decidedly more like the Chinese variety.

In addition to the measurements given in Tables II and III, there are a number of differences that deserve to be more fully discussed.

## HAIRS ON THE LEAF SHEATH

Perhaps the most striking difference between the varieties is the covering of the leaf sheaths. In the Chinese variety the leaf sheaths are similar to those of the ordinary types of maize. The surface is smooth, except for fine spicules, which occur especially over the fibrovascular bundles. The spaces between the fibrovascular bundles are crossed by numerous diagonal ridges or cross veins irregularly arranged and usually discontinuous at the fibrovascular bundles. These cross veins with the fibrovascular bundles cover the surface of the sheath with a coarse reticulum.

In the Esperanza variety the cross veins of the sheaths are absent or confined to the seedling leaves, and the spaces between the bundles are occupied by tubercles, each bearing a long hair (Pl. LVIII). These tuberculate hairs are absent from the sheath of the first six to eight leaves of the seedling. They appear abruptly and may cover the entire surface of the first sheath on which they appear. The hairs are from 3 to 5 mm. long, and the tubercle is approximately ½ mm. wide and of the same height.

In the Waxy Chinese variety tuberculate hairs are completely absent (Pl. LVI, fig. 2). As in all varieties, there is a small area closely confined to the throat of the sheath that is clothed with long hairs. It is not clear whether these hairs are homologous to the tuberculate hairs of the Esperanza variety or not. Even considering these hairs at the throat of the leaf sheath in the Waxy Chinese variety as of the same type, the two varieties are completely separated, with not even an approach to overlapping forms. In the hybrid and its progeny three methods of measuring the degree of hairiness were employed:

(1) By recording the total number of nodes with hairy sheaths.

(2) By recording the number of nodes with hairs completely encircling the sheath. In the pure Esperanza maize this usually occurred at the lowest node on which hairs were borne; or at most there was a difference of only one of two nodes. In the hybrid plants there were usually a number of sheaths with tuberculate hairs at the side, but with a narrow smooth strip at the back over the midrib.

(3) By recording the length of the longest tuberculate hairs. In all hybrid plants of both the first and second generation tuberculate hairs were present, there being no plant that resembled pure Waxy Chinese plants in this particular. The length of the hairs varied, however, in different plants, thus affording another measure of the extent to which hairs were developed.

## TASSEL CHARACTERS

In the nature of the tassel the two varieties are hardly less distinct than in the covering of the leaf sheath. The Waxy Chinese variety has many branches, 15 to 30 primary branches in normally developed plants, with numerous secondaries. The Esperanza (Pl. LVII) seldom has more than 5 branches and in many plants the tassel is simple, consisting only of a large central spike. Associated with the difference in the number of branches is a corresponding difference in length of the axis or "branching space," the distance from the lowest to the uppermost branch.

In the Esperanza variety the glumes vary from 10 to 16 mm. in length with a mean of $11.7 \pm 0.14$. In the Waxy Chinese variety the range is from 7 to 12 mm., with a mean of $9.2 \pm 0.09$. All of the above characters were directly measured or counted.

The typical arrangement of the spikelets is also different in the two varieties. In the Waxy Chinese the arrangement on the branches is similar to that in most of the better known varieties of maize. The spikelets are paired, one pediceled and one sessile, the pairs alternating on the sides of the branch. In the Esperanza maize when branches occur the spikelets are nearly all sessile and are borne in clusters of from 2 to 5. They are also disposed on all faces of the branch instead of being confined to the sides. The arrangement of spikelets and general appearance of the branches in the Esperanza is similar to the arrangement on the central spike. One result of these differences in arrangement of spikelets is a greater crowding of spikelets in the Esperanza. As a measure of this difference the number of spikelets in the last 10 cm. of the lowest tassel branch were counted. This number is referred to as the "density of the spikelets."

## TASSEL EXSERT

In the Waxy Chinese variety the base of the tassel is frequently inclosed in the uppermost leaf sheath. In the Esperanza variety the lowest branch of the tassel is usually well above the uppermost leaf

sheath in the mature plant. Differences in this particular were recorded by measuring the distance from the top of the uppermost sheath to the origin of the lowest tassel branch, the measurement being expressed as a minus quantity when the base of the branch was included in the sheath.

This character is especially subject to environmental changes. Unfavorable conditions, such as drought occurring late in the season, will prevent the elongation of the upper internodes to such an extent that all varieties may show a minus exsert. Comparisons must therefore be confined to plants grown in a single season in the same locality.

The range as recorded for Waxy Chinese grown at different times is from $-14$ cm. to 7 cm., with the mean at $-1.31 \pm 0.3$. In Esperanza the range is from $-3$ cm. to 18 cm., with the mean at $6.07 \pm 0.5$.

## NUMBER OF ERECT LEAF BLADES

In the Waxy Chinese variety the upper leaf blades are held erect instead of diverging. In ordinary varieties which the Esperanza resembles with respect to this character the upper leaf blades make approximately a right angle with the axis (Pl. LV, LVII). As a measure of this character the number of erect leaf blades was recorded. For example, if the two uppermost leaves were erect and the third leaf was the first to exhibit an angle, the plant was classed as 2, with respect to this character.

Recorded in this way there would be some overlapping in the parent varieties, since in some Waxy Chinese plants even the uppermost leaf shows an appreciable angle. In reality, however, the two types are distinct, for in the Esperanza not only is the uppermost leaf never erect, but it is seldom borne at less than a right angle with the stalk.

## ANGLE OF TASSEL AXIS

In the Esperanza variety the tassel is always erect. In the Waxy Chinese plant the tassel is usually curved or declined (Pl. LV, LVII). This character is variable in the Chinese, some plants having the tassel perfectly erect. The tendency, however, to an inclined tassel, as it appears in the hybrid, may properly be ascribed entirely to the Chinese variety, no similar tendency ever having been observed in any Esperanza plant. The character was measured by estimating the angle which the branching space, or that portion of the axis of the tassel between the lowest and highest branch, made with the main stalk. In the pure Waxy Chinese variety this character appears definitely associated or physiologically correlated with the following character of "one-sidedness."

## ONE-SIDEDNESS

One of the most striking peculiarities of the Waxy Chinese variety of maize is the displacement of the leaf blades from the usual distichous arrangement, with the result that a number of the upper leaf blades are

borne on one side of the plant instead of alternately on opposite sides of the culm (Pl. LVI, fig. 1). Like the angle of the tassel, this character is not universally present in the Waxy Chinese plant, but, on the other hand, no tendency of this kind has ever been observed in the Esperanza variety.

When one-sided plants occur in the hybrid generations, it is therefore reasonable to assume that the character was derived from the Chinese parent. Measurements of these characters in the hybrid plants were made by recording the number of monostichous or single-ranked leaves.

A recapitulation of the more definitely contrasting characters of the two parent varieties is here presented in parallel columns:

| Esperanza variety | Waxy Chinese variety |
|---|---|
| Horny endosperm. | Waxy endosperm. |
| Branching space short. | Branching space long. |
| Tassel erect. | Tassel curved. |
| Spikelets in clusters. | Spikelets in pairs. |
| Glumes long. | Glumes short. |
| Leaf sheaths with tuberculate hairs. | Leaf sheaths without tuberculate hairs. |
| Upper leaf blades horizontal. | Upper leaf blades erect. |
| Upper leaf blades distichous. | Upper leaf blades monostichous. |

If the characters of maize were subject to coherence, the second generation of a cross between two such diverse and long-established types as Esperanza and Waxy Chinese would seem a most favorable opportunity for its manifestation.

In the whole series of second-generation plants there were none that even approximately represented either parent variety; nor did the plants fall into recognizable groups. With respect to the individual characters, the parental forms reappeared or were even intensified in some instances, but an almost complete and chance reassortment of the characters seems the rule. If the characters were completely independent, a reappearance of the parental types could not, of course, be expected, for, treating the characters as alternative and allowing for only 10 characters, a plant possessing all the characters of either parent could not be expected oftener than once in 10 billion plants. Although the characters themselves, with few exceptions, were non-Mendelian in the sense that they were not alternative, the results conformed to the Mendelian law of recombination. Examples of the combination of characters from the two parent varieties are shown in Plates LIX to LXIII.

Endosperm texture was the only strictly alternative character noted. The number of erect leaves and angle of tassel, while not alternative in the sense of falling into definite groups without intermediates, do, however, approach a Mendelian form of inheritance. The distribution, instead of approximating a normal frequency curve, was distinctly bimodal with respect to these characters. A similar tendency is apparent in the first-generation plants. In connection with this evidence

of segregation in the first generation, it should be recalled that neither of these characters, which belong to the Waxy Chinese variety, is universally present in the plants of that variety, and the parent plant may have been heterozygous. There is also a less pronounced indication in the second-generation plants that one-sideness is Mendelian in its inheritance.

## CORRELATIONS

Eleven of the characters most definitely contrasted in the parents were selected and the correlation coefficients between all possible combinations were calculated for both the first and second-generation plants. The results are shown in Table IV. The correlations are so stated that a positive, or plus, correlation indicates a correlation between the characters derived from the same parent; in other words, a coherence. For example, the Waxy Chinese variety has a large number of tassel branches and no tuberculate hairs, while the Esperanza variety has a small number of tassel branches and well-developed tuberculate hairs. In expressing the relation between these two characters, when a large number of tassel branches is found associated with short tuberculate hairs, the correlation is recorded as positive.

Since ears 1 and 2 were reciprocals and no significant differences were found between their progenies, the observed values were used directly in calculating the coefficients of correlation. Where the mean progeny of ear 3 differed from the mean of the combined progenies of ears 1 and 2 with respect to any character, all measurements in the progeny of ear 1 were multiplied by the percentage difference between the means before combining the populations in a correlation table.

The combined progenies of the three first-generation ears numbered 183 individuals. Complete notes could not be taken on all the plants, so that the number of individuals entering into the different correlation tables was reduced to from 125 to 150. Assuming all correlations that are more than 3.5 times the probable error to be worthy of consideration, an examination of Table III shows that 20 of the 55 character pairs fall into this class.[1] With three exceptions the coefficient for the character pairs of this group is 0.2 or larger. Of these 20 character pairs that may be held to show definite correlations in the second generation, 17 are positive—that is, in the nature of coherences—and 3 are negative. All but 5 of the 20 are, however, open to the suspicion of being physiological correlations, since they do not differ materially from the correlations shown for the same characters in the first generation.

The 5 character pairs that show most evidence of genetic correlation are given in Table V. Even here there are no very striking differences between the coefficients of the first and second generations, and it is by no means impossible that even here the differences may be due to chance.

[1] These coefficients are printed in bold-face type in Table IV.

TABLE IV.—*Correlation coefficients*

| Characters. | First generation. | | Second generation. | | |
|---|---|---|---|---|---|
| | Coef. | P.E.[1] | Coef. | P.E.[1] | Coef. / P.E. |
| Small exsert of tassel and long branching space | 0.27 | 0.13 | 0.345 | 0.053 | 6.5 |
| Small exsert of tassel and large number of tassel branches | .30 | .13 | .287 | .055 | 5.2 |
| Small exsert of tassel and large number of erect blades | .22 | .14 | .346 | .059 | 5.9 |
| Small exsert of tassel and high degree of one-sidedness | .00 | .15 | .353 | .056 | 6.3 |
| Small exsert of tassel and large angle of tassel axis | .18 | .14 | .411 | .050 | 8.2 |
| Small exsert of tassel and small number of sheaths with hairs | −.21 | .13 | −.109 | .059 | 2.0 |
| Small exsert of tassel and short hairs | −.39 | .12 | −.150 | .060 | 2.5 |
| Small exsert of tassel and low density of spikelets | −.10 | .14 | .018 | .059 | .3 |
| Small exsert of tassel and short glumes | −.24 | .13 | −.036 | .059 | .6 |
| Small exsert of tassel and waxy endosperm | ... | ... | −.069 | .070 | .9 |
| Long branching space and large number of tassel branches | .50 | .09 | .442 | .045 | 9.8 |
| Long branching space and large number of erect leaf blades | −.22 | .14 | .202 | .061 | 3.3 |
| Long branching space and high degree of one-sidedness | −.14 | .13 | .234 | .057 | 4.1 |
| Long branching space and large angle of tassel axis | .00 | .13 | .170 | .055 | 3.1 |
| Long branching space and small number of sheaths with hairs | .00 | .12 | .091 | .054 | 1.7 |
| Long branching space and short hairs | −.12 | .12 | .056 | .056 | 1.0 |
| Long branching space and low density of spikelets | .32 | .11 | .198 | .054 | 3.7 |
| Long branching space and short glumes | −.01 | .12 | −.188 | .053 | 3.6 |
| Long branching space and waxy endosperm | ... | ... | .242 | .066 | 3.7 |
| Large number of tassel branches and large number of erect blades | −.11 | .14 | .222 | .063 | 3.5 |
| Large number of tassel branches and high degree of one-sidedness | .27 | .12 | .221 | .058 | 3.8 |
| Large number of tassel branches and large angle of tassel axis | .38 | .11 | .243 | .054 | 4.5 |
| Large number of tassel branches and small number of sheaths with hairs | −.30 | .11 | −.091 | .056 | 1.6 |
| Large number of tassel branches and short hairs | .25 | .12 | −.021 | .057 | .4 |
| Large number of tassel branches and low density of spikelets | .45 | .10 | .214 | .054 | 4.0 |
| Large number of tassel branches and short glumes | .07 | .12 | −.165 | .054 | 3.0 |
| Large number of tassel branches and waxy endosperm | ... | ... | .104 | .067 | 1.6 |
| Large number of erect leaf blades and high degree of one-sidedness | .17 | .14 | .487 | .050 | 9.7 |
| Large number of erect leaf blades and large angle tassel axis | .29 | .13 | .513 | .047 | 10.9 |
| Large number of erect leaf blades and small number of sheaths with hairs | −.21 | .14 | −.118 | .064 | 1.8 |
| Large number of erect leaf blades and short hairs | .17 | .14 | −.081 | .063 | 1.3 |
| Large number of erect leaf blades and low density of spikelets | −.18 | .14 | .084 | .065 | 1.3 |
| Large number of erect leaf blades and short glumes | .31 | .13 | .043 | .065 | 1.1 |
| Large number of erect leaf blades and waxy endosperm | ... | ... | .... | .081 | ... |
| High degree of one-sidedness and large angle of tassel axis | .67 | .07 | .582 | .040 | 14.5 |
| High degree of one-sidedness and small number of sheaths with hairs | −.15 | .13 | −.069 | .059 | 1.2 |
| High degree of one-sidedness and short hairs | .15 | .13 | .068 | .060 | 1.1 |
| High degree of one-sidedness and low density of spikelets | .36 | .12 | .030 | .059 | .5 |
| High degree of one-sidedness and short glumes | −.08 | .13 | −.017 | .060 | .3 |
| High degree of one-sidedness and waxy endosperm | ... | ... | .118 | .074 | 1.6 |
| Large angle tassel axis and small number of sheaths with hairs | −.23 | .11 | −.203 | .053 | 3.8 |
| Large angle tassel axis and short hairs | .01 | .12 | .007 | .056 | .1 |
| Large angle tassel axis and low density of spikelets | .02 | .13 | .123 | .055 | 2.2 |
| Large angle tassel axis and short glumes | .47 | .10 | .043 | .056 | .8 |
| Large angle tassel axis and waxy endosperm | ... | ... | .037 | .079 | .5 |
| Small number of sheaths with hairs and long hairs | −.05 | .12 | .193 | .053 | 3.6 |
| Small number of sheaths with hairs and low density of spikelets | −.01 | .12 | −.134 | .058 | 2.3 |
| Small number of sheaths with hairs and short glumes | .21 | .11 | −.009 | .055 | .2 |
| Small number of sheaths with hairs and waxy endosperm | ... | ... | .192 | .067 | 2.9 |
| Short hairs and low density of spikelets | .12 | .12 | −.027 | .055 | .5 |
| Short hairs and short glumes | −.05 | .12 | .013 | .055 | .2 |
| Short hairs and waxy endosperm | ... | ... | −.014 | .069 | .2 |
| Low density of spikelets and short glumes | −.16 | .12 | −.233 | .052 | 4.5 |
| Low density of spikelets and waxy endosperm | ... | ... | .082 | .068 | 1.2 |
| Short glumes and waxy endosperm | ... | ... | .177 | .059 | 3.0 |

[1] P. E.=probable error.

TABLE V.—*Character pairs exhibiting genetic correlations*

| Character pair. | Coefficient of correlation. | | Difference between first and second generations. | Difference÷probable error. |
|---|---|---|---|---|
| | First generation. | Second generation. | | |
| Small exsert of tassel and one-sidedness | 0.00±0.15 | 0.353±0.056 | 0.353±0.160 | 2.2 |
| Branching space and number of erect blades | −.22± .14 | .202± .061 | .422± .153 | 2.8 |
| Branching space and one-sidedness | −.14± .13 | .234± .057 | .374± .142 | 2.6 |
| Number of tassel branches and number erect | −.11± .14 | .222± .063 | .332± .143 | 2.3 |
| Number of erect blades and one-sidedness | .17± .14 | .487± .050 | .317± .149 | 2.1 |

Owing to the small number of first-generation individuals and the consequent uncertainty that attaches to correlation coefficients in that generation, it is, on the other hand, possible that other correlations shown in the second-generation plants are really genetic. From this point of view, it should be noted, however, that 18 of the second-generation correlations are negative.

The possibility of a reduction of physiological correlations must also be considered. The existence of a significant positive correlation in the first generation is taken to indicate a physiological correlation between the characters. With such characters as branching space and the number of branches, the relation is obvious; indeed this relation might almost be classed as physical, since as the branching space approaches zero the number of branches must necessarily become less. There would also appear to be a necessary relation between one-sidedness and angle of the tassel axis, for a perfectly erect tassel could scarcely occur with a high degree of one-sidedness. Where correlations of this nature are lowered in the second generation, it would seem necessary to assume that this reduction is brought about by a tendency for the characters from *different* parents to reappear in the same individual, thus reducing the normal physiological correlation that exists between the characters.

The following are two such character pairs:

| | First generation | Second generation | Difference | $\frac{D.}{P.E.}$ |
|---|---|---|---|---|
| One-sidedness and low density of spikelets | 0.36±0.12 | 0.030±0.059 | 0.330±0.13 | 2.5 |
| Large angle of tassel axis and short glumes | .47± .10 | .043± .056 | .427± .114 | 3.7 |

It has been mentioned that with respect to both the number of erect leaf blades and the angle of tassel axis there was a tendency for the plants to fall into two groups. This raised a doubt as to the applicability of the customary "product-moment" method of calculating the correlation coefficient where these characters were involved. This group of correlations was therefore recalculated, using Pearson's biserial correlation coefficient (Pearson, 1909). Slightly different values were obtained, but no additional significant correlations were brought to light.

In the second generation the waxy and horny seed were planted separately, thus affording an opportunity for observing whether the plants from seeds having the waxy endosperm characteristic of the Waxy Chinese variety showed any preponderance of other Chinese characters. No consistent differences were apparent in the general appearance of the rows from the waxy and horny seeds. There was such great individual diversity, however, that comparison was difficult. Analysis of the measurements showed little more. The only character that showed a measurable correlation with endosperm texture was the degree to which tuberculate hairs were developed on the leaf sheaths.

Since endosperm texture is strictly alternative, while all other characters were expressed in varying degrees, the method for calculating the correlation coefficients was necessarily different for this group of character pairs. In calculating the correlations with endosperm texture Pearson's (1909) method for calculating a biserial correlation, together with Soper's (1914) formula for the probable error, were used. With a strictly alternative character such as endosperm texture, it would seem impossible to distinguish physiological from genetic correlations. Since one variety always has waxy and the other always has horny endosperm, to detect correlations with this character in the parent varieties seems out of the question. Likewise, as a result of the dominance of the horny endosperm, the seeds from which the first-generation plants were grown were all horny, and there was no opportunity to determine correlations with endosperm texture among first-generation plants.

At the time of planting it was, of course, impossible to distinguish between the seed that were pure for the horny character and those that were heterozygous. An examination of the open-pollinated ears produced by the second-generation plants grown from horny seeds made such a separation possible. All ears that produced any waxy seeds must have grown from heterozygous seeds. No correlations sufficiently large to be detected in the small number of individuals available were found between these two classes and other contrasting characters.

It may be urged that the absence of coherence in the progeny of such a diverse hybrid as the one here discussed may not prove that there is a similar lack of coherence among crossbred individuals within the variety. All maize varieties are, however, of such mixed ancestry that they are in effect hybrid progenies, and even if an exhaustive study of the inheritance of the characters of a narrow-bred variety should show the existence of coherence the results would be beside the point from a practical standpoint, for to maintain a satisfactory degree of vigor in maize a condition of mixed ancestry must be retained.

## INTENSIFICATION OF CHARACTERS

The present hybrid affords an interesting sample of an intensified character. One of the peculiarities of the Waxy Chinese variety is the scorpioid top. In plants which exhibit this character the leaf blades of the upper nodes are monostichous and erect, and the tassel is curved to one side. The curving of the tassel was originally interpreted as a direct result of the monostichous arrangement and erect blades. The manner in which this complex of characters reappears in the hybrid with the Esperanza variety shows that, although always associated in pure Chinese maize, they are separable and each may be inherited independently of the others. The curved tassel supposed to be merely the result of the other characters may not only occur alone—that is, in plants with

distichous leaf blades all of which make an angle with the main axis—but the extent of the curving is much greater in some of the hybrid plants than has ever been observed in pure Waxy Chinese plants. The angle of the tassel axis had not been recorded for Waxy Chinese plants before the season of 1915, but thousands of individuals have been observed, and it can be definitely stated that no plant showed a tassel inclined as much as 90° from the perpendicular.

In 148 hybrid plants of the second generation of the hybrid there were 12 plants with the axis of the tassel inclined from the perpendicular by more than 100° and 5 plants having the angle of the tassel axis recorded as more than 145°. The phenomenon is not due to any weakness of the culm, as examples of more than 180° show (Pl. LXII); in fact, the upper part of the culm is particularly thick and rigid, a characteristic of the Chinese parent.

The positiveness of the character was well shown in some of the plants where the curving of the culm caused it to break through the upper leaf sheaths. In such plants the pendent tassels very strongly suggested the "goose neck" of certain sorghum varieties. A plant of this type is shown in Plate LXIII.

## CONCLUSIONS

Two principal methods of breeding may be distinguished, depending on the manner in which selection is applied:

(1) Selection may be directed toward the isolation and propagation of desirable types of individuals. The new type may occur as an aberrant individual or as a recognizably distinct strain within the variety, but in either case it is differentiated from the stock by many characters.

(2) Selection is directed to variations of the individual characters regarding which improvement is desired.

With most crop plants the method of selecting types has been by far the most productive, but in the improvement of maize, this method has figured very little. Selection has been by characters instead of by types.

Why the isolation of types of plants has not been a factor in the improvement of maize has not been clear. Though diversities in plant characters are obvious and striking, few breeders have been able to distinguish well-defined types of plants within commercial varieties.

If recognizable types exist it must mean that groups of characters tend to appear together; in other words, the characters are correlated. The extent to which obvious characters are correlated is therefore proposed as a measure of this tendency toward the persistence of types. In the progeny of a hybrid between two very different maize varieties the results here reported show that the characters studied, instead of forming coherent groups, are almost completely independent in inheritance.

By attempting to measure the extent to which types persist by means of correlation coefficients, it is necessary to distinguish different kinds of correlations. For this purpose correlations are here classified as physical, physiological, and genetic. A method is also proposed by which physiological and genetic correlations may be distinguished.

The case studied was a hybrid between two extreme types that must have been completely isolated from very remote times. The large number of well defined characters which differentiate the varieties rendered this material exceptionally favorable for the study of coherence, by which is meant the tendency for characters associated in one of the parents of a hybrid to remain together in the later generation of the hybrid.

For the study of correlations 11 characters were selected in which the parent varieties showed little or no overlapping. The correlation coefficients of all the combinations were calculated, and of the 55 possible combinations 20 were found to exhibit significant correlations. In all but 5 of these, however, the correlations are believed to be physiological rather than genetic. In no instance was there a correlation between two characters closer than 0.5, a fact which in itself offers an explanation of the difficulty of recognizing types in maize.

This lack of coherence of characters in maize, taken with the fact that to maintain a satisfactory degree of vigor a diversified ancestry must be maintained, would appear to make the method of isolating types inapplicable to this plant. As an offset to the limitation thus imposed, advantage may be taken of the facility with which desirable characters derived from different parents can be combined.

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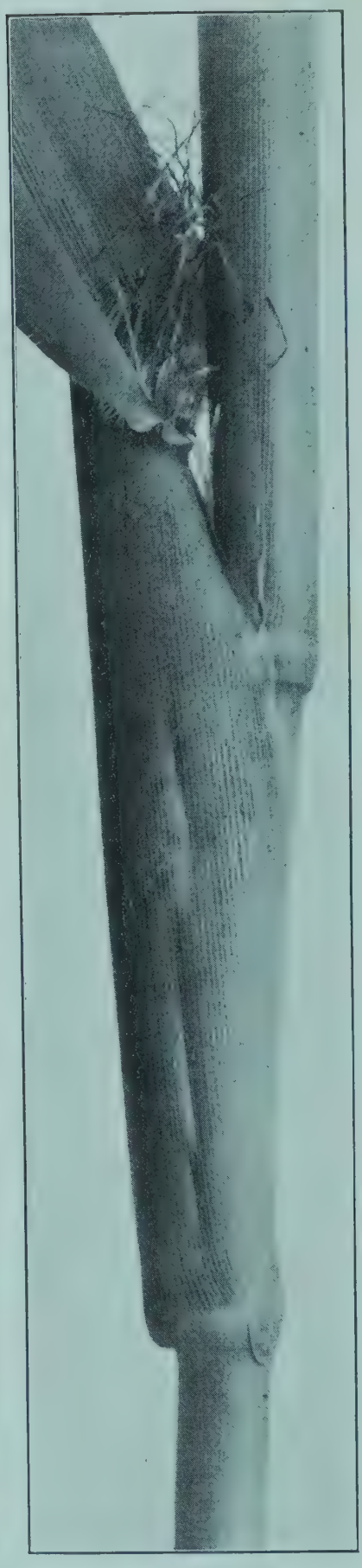
## PLATE LV

Typical plant of the Waxy Chinese variety of maize, showing numerous tassel branches, erect leaf blades, one-sidedness, and curved tassel.

1



2

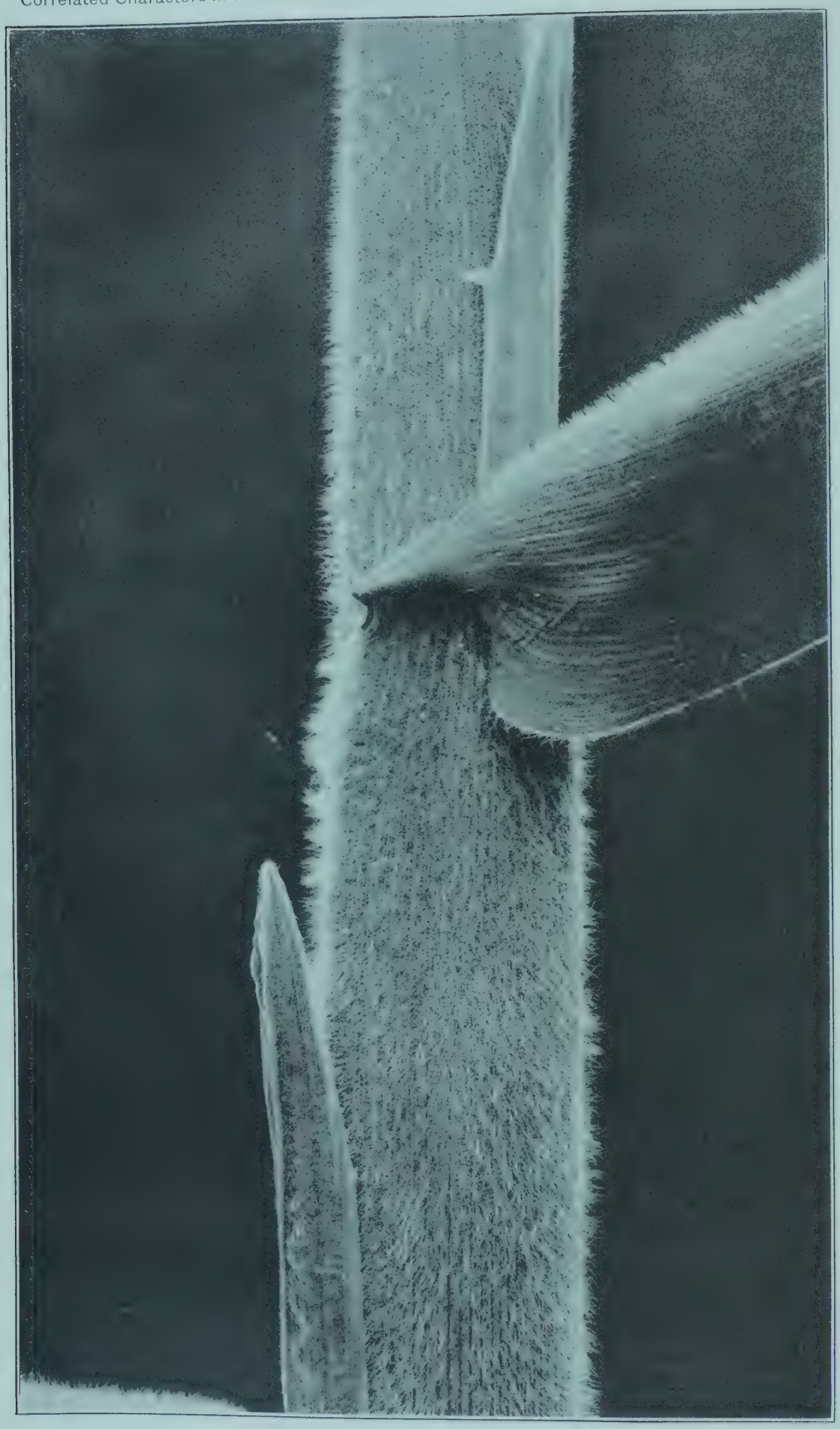
## PLATE LVI

Fig. 1.—Uppermost leaf sheaths of Chinese maize plant, showing the one-sided arrangement of leaf blades and absence of hairs. Natural size.

Fig. 2.—Leaf sheath of the Waxy Chinese variety of maize, showing the transverse lines and absence of hairs. Compare with Plate LX. Natural size.

## PLATE LVII

A plant of the Esperanza variety of maize, showing the drooping leaves, few tassel branches, and elongated internodes characteristic of the variety.



## PLATE LVIII

Leaf sheaths of the Esperanza variety of maize, showing the maximum development of tuberculate hairs. Compare with Plate LVI. Natural size.

## PLATE LIX

A leaf sheath of a second-generation hybrid maize plant. This plant represents the maximum length of hairs. They are even longer than any thus far observed in the Esperanza variety. Compare with Plate LX. Natural size.

## PLATE LX

A first-generation plant of Chinese × Esperanza maize hybrid. Measured by the number of sheaths with hairs, this was the most hairy plant in the first generation. Combined with this Esperanza character is an accentuation of the Chinese character of a scorpioid top.

## PLATE LXI

A second-generation plant of a Chinese×Esperanza maize hybrid. This plant showed a maximum development of the Esperanza character of hairiness combined with the erect crowded leaf blades and deflexed tassel of the Chinese variety.

## PLATE LXII

A second-generation plant of a Chinese×Esperanza maize hybrid. An extreme example of the scorpioid top; the angle was recorded as 190°.

## PLATE LXIII

A second-generation plant of a maize hybrid, showing the "goose-neck" character that appeared for the first time in this hybrid. This plant showed few Esperanza characters. Although the plant is one-sided, it shows that the displacement of the tassel is not the result of crowding by the leaf blades.

# COMPARATIVE STUDY OF THE AMOUNT OF FOOD EATEN BY PARASITIZED AND NONPARASITIZED LARVÆ OF CIRPHIS UNIPUNCTA

By DANIEL G. TOWER,

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## INTRODUCTION

The aim of an experiment which was conducted at the United States Entomological Laboratory in West La Fayette, Ind., during the summer of 1915, was to determine whether larvæ of the army worm (*Heliophila*) *Cirphis unipuncta* Haworth, when attacked by an internal parasite, *Apanteles militaris* Say, ate less, as much as, or were stimulated to eat more than when nonparasitized; and as a sequence, to determine whether this or a similar parasitism is directly beneficial in the generation parasitized or only indirectly, resulting in subsequent smaller generations. Although only 9 of the 25 parasitized larvæ with which the experiment was started lived until the emergence of the parasites, the others dying soon after oviposition took place, the records of these 9 larvæ are sufficiently definite to satisfy the purpose of the experiment.

The excellent work of Mr. J. J. Davis and Mr. A. F. Satterthwait [1] in determining the total amount of food eaten by healthy larvæ of *C. unipuncta* under different feeding conditions has been used to compare with the amount of food eaten by parasitized larvæ.

The results of the experiments have been drawn up in tabular form to show the life of the host larvæ from the time they were oviposited in until their death coincident with the emergence of the parasite and the life history of the parasite in relation to its host (Table I).[2]

## EXPERIMENTAL METHODS

The parasites were induced to oviposit in the host larva while confined in test tubes into which a larva was introduced and left until recognized as a host and parasitized. Often this occurred immediately, and three or four ovipositions might take place before the larva could be removed. In other cases it would be some minutes before the parasite could be induced to oviposit.

These parasitized larvæ were confined separately in large vials, placed in the shade in a well-aired room, and fed pieces of mature corn leaves, conveniently cut out so as to measure 1 square inch each.

In order to obtain unfertilized females, individual cocoons were placed in gelatin medicine capsules previous to the emergence of the adults, the sex being easily determined through the transparent gelatin, when the adults emerged.

[1] Data as yet unpublished; may appear in a later issue of this Journal.

[2] The author was ably assisted in the care and feeding of the larvæ by Mr. H. J. Hart, who was temporary assistant at the laboratory during the summer of 1915.

TABLE I.—*Life-history data relating to host larvæ of Cirphis unipuncta and to the parasites, Apanteles militaris, attacking them*

| Experiment No. | Width of head of host when oviposited in. | Age of host when oviposited in. | Date of ovipositions. | Number of ovipositions. | Duration of ovipositions in seconds. | Total amount of corn foliage eaten after parasitism. | Width of head of host when parasite emerged. | Age of host when parasite emerged. | Number of parasites in each host. | Date parasite spun cocoons. | Date adult parasite issued. | Time spent in host by parasite. | Time spent in cocoon by parasite. | Total life cycle of parasite. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mm.* | | 1915. | | | *Sq. in.* | *Mm.* | | | 1915. | 1915. | | | |
| 10 | 2.4 | Fifth instar... | Aug. 15, 1.32 p. m. | 3+ | 1 each..... | 16.21 | 3.4 | Sixth instar. | 49 | Aug. 29, previous to 8 a. m. | Sept. 7, previous to 8 a. m. | 13 d., 18 h., 28 m. | 9 d..... | 22 d., 18 h., 28 m. |
| 11 | 2.1 | .....do..... | Aug. 15, 1.37 p. m. | 2+ | 1 or less each.. | 12.16 | 3 | ...do..... | 39 | Aug. 29, between 10.30 a. m. and 6 p. m. | Sept. 8, previous to 8 a. m. | 13 d., 20 h., 53 m. to 14 d., 4 h., 23 m. | 9 d., 21 h., 30 m. to 9 d., 14 h. | 23 d., 18 h., 23 m. |
| 15 | 2.1 | .....do..... | Aug. 15, 1.50 p. m. | 1 | 1..... | 11.97 | ..... | ...do..... | 48 | Aug. 29, previous to 8 a. m. | Sept. 7, between 8 and 11.30 a. m. | 13 d., 18 h., 10 m. | 9 d. to 9 d., 3 h., 30 m. | 22 d., 18 h., 10 m. to 22 d., 21 h., 40 m. |
| 16 | 2.2 | .....do..... | Aug. 15, 2.03 p. m. | 1+ | 1 each..... | 14.50 | 3.2 | ...do..... | 63 | .....do..... | Sept. 7, previous to 8 a. m. | 13 d., 17 h., 57 m. | 9 d..... | 22 d., 17 h., 57 m. |
| 19 | 1.8 | Fourth instar. | Aug. 15, 2.55 p. m. | 2 | .....do..... | 20.63 | 3.5 | ...do..... | 100 | .....do..... | Sept. 8, previous to 8 a. m. | 13 d., 17 h., 5 m. | 10 d..... | 23 d., 17 h., 5 m. |
| 22 | 1.7 | .....do..... | Aug. 15, 3.06 p. m. | 3 | About 2 each.. | 17.36 | ..... | ...do..... | 123 | .....do..... | .....do..... | 13 d., 16 h., 54 m. | 10 d..... | 23 d., 16 h., 54 m. |
| 23 | 1.8 | .....do..... | Aug. 15, 3.12 p. m. | 3 | .....do..... | 21.41 | 3.6 | ...do..... | 154 | Aug. 31, previous to 7 a. m. | Sept. 8, between 8 and 10.30 a. m. | 15 d., 15 h., 48 m. | 8 d., 1 h. to 8 d., 3 h., 30 m. | 23 d., 16 h., 48 m. to 23 d., 19 h., 18 m. |
| 24 | 1.8 | .....do..... | Aug. 15, 3.20 p. m. | 3 | .....do..... | 17.64 | 3.4 | ...do..... | 88 | Aug. 29, previous to 8 a.m. | Sept. 8, previous to 8 a. m. | 13 d., 16 h., 40 m. | 10 d..... | 23 d., 16 h., 40 m. |
| 25 | 1.7 | .....do..... | Aug. 15, 3.30 p. m. | 3+ | 1+ each..... | 17.99 | 3.3 | ...do..... | 98 | ..... | Sept. 9, previous to 8 a. m. | ..... | ..... | 24 d., 16 h., 30 m. |
| Average..... | | | | | | | | | | | | 14 d., 11½ m.. | 9 d., 8 h., 45 m. | 23 d., 12 h., 26 m. |

## LIFE CYCLE OF THE PARASITE

The biology of *A. militaris* has already been studied and the results published.[1]

Oviposition took place with great rapidity and apparently anywhere in the host, attempts even being made by the parasite to oviposit in the head. The largest number of eggs inserted at one time, according to the observations herein recorded, was 154 for 3 ovipositions, averaging 51 + each (Table I, Experiment 23). The two endoparasitic stages and the egg stage required an average of 14 days, 11½ minutes, while the time spent by the third larval stage and the pupal stage in the cocoon averaged 9 days, 8 hours, and 45 minutes, and the average for the total life cycle was 23 days, 12 hours, and 26 minutes.

The parasitic larva leaves its host by means of an individual exit hole cut through the muscles and epidermis by its mandibles. As the larvæ squeeze through the holes they molt their second larval skins, and when about two-thirds of their way out commence to spin their cocoons. After the cocoon is spun and previous to pupation, the accumulated wastes are passed, being deposited at one end of the cocoon. Shortly following this the larva pupates and the last larval skin is pushed to the same end of the cocoon.

The adult issues, after kicking off its pupal skin, by cutting off a cap-like portion at one end of the cocoon, cleans itself, and at the same time passes a quantity of waste. It is now ready for copulation, oviposition, or feeding, as the case may be. In this respect it was found that females were at once ready to oviposit following emergence and previous to feeding or copulation, and that the progeny from such females were all males. Hence it is seen that unfertilized females give rise parthenogenetically to a generation of males.

## CONCLUSIONS

In using the data compiled by Davis and Satterthwait on the amount of food eaten by healthy larvæ of *C. unipuncta*, for comparison with the amount eaten by parasitized larvæ, it will only be necessary to use the feeding records for the last three instars in one series of their experiments, this being the one in which the larvæ were confined in lantern-globe cages. These records were selected in preference to those obtained by keeping the larvæ in large vials, because in the former case a larger number of records were obtained, although in the latter case the averages of the feeding records for the same periods run higher.

Larvæ 10, 11, 15, and 16 were newly molted fifth-stage specimens when oviposited in, and they ate 16.21, 12.16, 11.97, and 14.50 square inches of corn foliage, respectively, during their last two stages previous to the emergence of the parasites, which is a much smaller amount than

[1] Tower, D. G. Biology of Apanteles militaris. *In* Jour. Agr. Research, v. 5, no. 12, p. 495–508, 1 fig., pl. 50. 1915.

the average of 33.6 square inches eaten by 20 nonparasitized larvæ during the same stages. Larvæ 19, 22, 23, 24, and 25 were partially developed fourth-stage specimens when oviposited in, and they ate, during the remainder of their life, which lasted until the parasites emerged from them some time during the last or sixth stage, 20.63, 17.36, 21.41, 17.64, and 17.99 square inches of corn leaf, respectively, as compared with the average of 34.77 square inches eaten by 20 nonparasitized larvæ during the last three stages. (See Table I.)

From these results it will be seen that parasitized larvæ ate approximately half as much as unparasitized larvæ during the same periods, and it seems conclusive, even from these few records, that parasitism by *A. militaris* is directly beneficial in the generation attacked. From the results obtained it might seem as though larvæ oviposited in at an earlier date would eat more before being killed, but the time spent in the host by the parasites seems to be fairly constant, and this was also noticed in a larger number of cases in former experiments with *A. militaris*. Hence, it is believed that in such cases the larvæ would have only approximately the same amount of time for feeding, and a larger portion of this period would occur during the earlier stages, when a much smaller amount of food is eaten, so that the amount eaten would be less than the normal for unparasitized larvæ.

# ALEYRODIDAE, OR WHITE FLIES ATTACKING THE ORANGE, WITH DESCRIPTIONS OF THREE NEW SPECIES OF ECONOMIC IMPORTANCE

By A. L. QUAINTANCE, *Entomologist in Charge of Deciduous Fruit Insect Investigations*, and A. C. BAKER, *Entomological Assistant, Bureau of Entomology*

Thirteen species of so-called white flies are recorded in literature as infesting Citrus plants in different parts of the world. Eight of these are present in Florida, four of them being native to the United States and four having been introduced. The native forms have thus far been of little economic importance, whereas two of the introduced species are first-class Citrus pests. The remaining two introduced forms, although recently established on the orange (*Citrus aurantiaca*), have already attracted attention by reason of their injuries. Our knowledge of the remaining five species of Citrus white flies, while meager, indicates that these, in their range of distribution, are abundant and destructive and would in all probability prove to be very undesirable immigrants. The new forms treated herein must be classed in the same category, especially *Aleurocanthus woglumi*, which, although previously named, is here technically described for the first time. This last species, of oriental origin, has already found its way to Jamaica and the Bahamas, where it infests the orange to a serious extent.

The present paper brings together the essential information concerning the distribution and food plants of the white flies which attack Citrus plants and describes three new species of economic importance.

**Aleurocanthus citricolus** (Newstead)

*Aleurodes citricola* Newst., 1911, *in* Mitt. Zool. Mus. Berlin, Bd. 5, Heft 2, p. 173.[1]

This species is known only from the original description. It was taken at Dar es Salaam, German East Africa, on *Citrus* sp. in 1902. The immature stages occurred in large, overcrowded colonies, appearing to the unaided eye as patches of a sootlike deposit on the lower surface of the leaves. This is one of the spiny forms and bears a general resemblance to *A. woglumi* (fig. 2, *A–J*, Pl. LXIV, LXV).

**Aleurocanthus citriperdus**, n. sp.

This insect (fig. 1) was taken by Mr. R. S. Woglum, of the Bureau of Entomology, in several localities in the Orient, as follows: Royal Botanic Gardens, Ceylon, on an unknown tree, October, 1910; Lahore, India, on *Citrus* sp., July, 1911; Buitenzorg, Java, on orange, January, 1911; Sandan Glaya, Java, on *Citrus* sp., January, 1911. It is reported

[1] All bibliographic citations in synonymy are given in full in "Literature cited," pp. 471–472.

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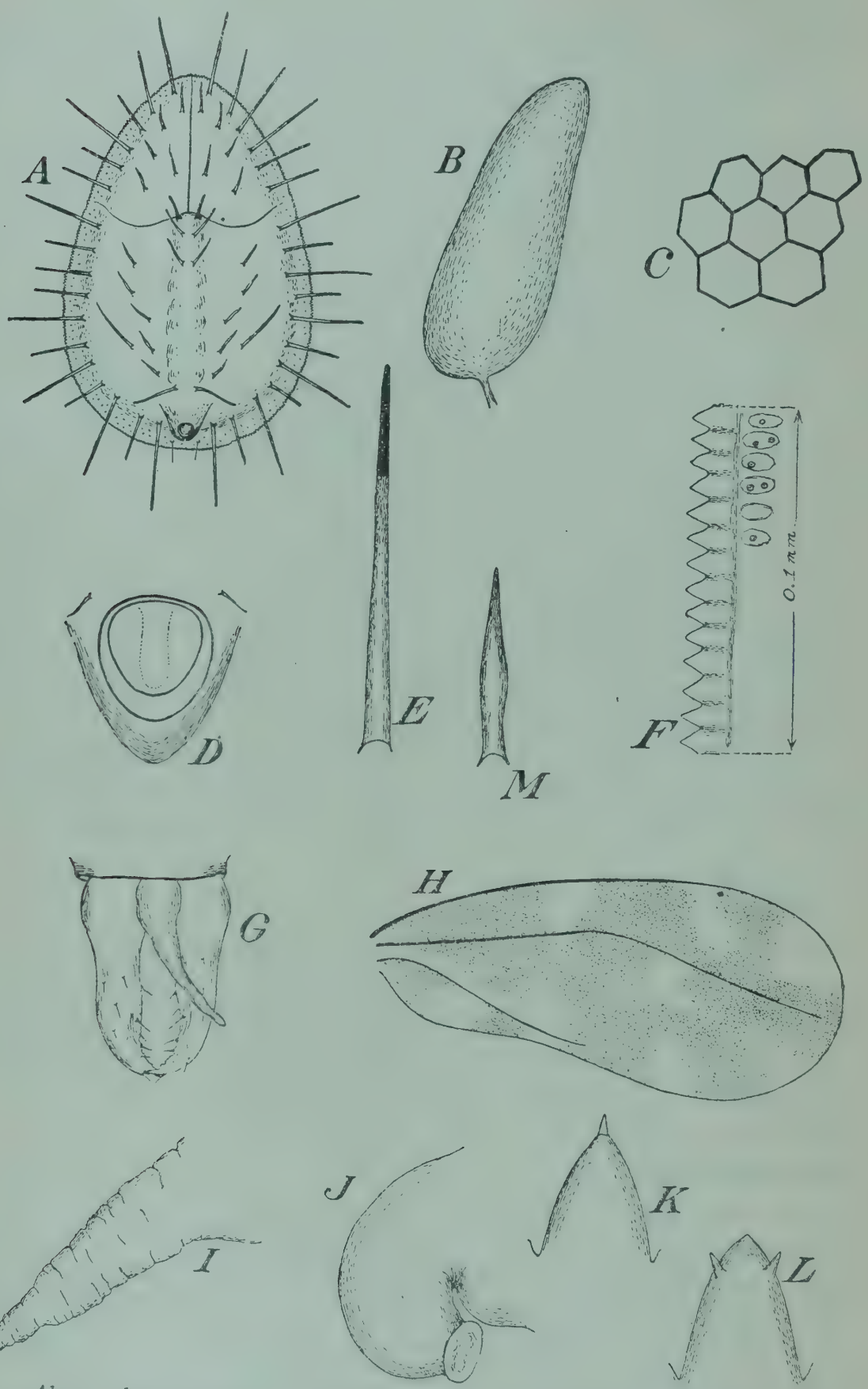


FIG. 1.—*Aleurocanthus citriperdus:* *A*, Pupa case; *B*, egg; *C*, polygonal markings of egg; *D*, vasiform orifice of pupa case; *E*, spine from dorsum of pupa case; *F*, margin of pupa case; *G*, genitalia of adult male; *H*, forewing of male; *I*, antenna of pupa case; *J*, leg of pupa case; *K*, *L*, marginal teeth, much enlarged; *M*, central swollen spine from dorsal area.

as occurring abundantly on species of Citrus and is regarded as of considerable economic importance.

PUPA CASE (fig. 1, *A*).—Length 1.36 mm.; width 0.96 mm.; shape elliptical to oval, broadest across the third abdominal segment, narrowest cephalad. Dorsum with a moderate central abdominal ridge on which the abdominal segments are not distinctly marked off, though they may be distinguished. Submarginal area somewhat flat; suture separating the thorax and abdomen quite distinct; surface appearing somewhat granular or faintly corrugated, an appearance which may be due to difference in pigmentation. Dorsum with numerous heavy spines (fig. 1, *E*) which after clearing remain black at the tips, but are otherwise a clear greenish yellow. These are arranged as follows: On the submarginal area is a more or less even row of usually 32 spines. This row is composed of two series alternating with one another. The one is made up of spines averaging about 0.288 mm., and the other of spines averaging 0.192 mm. in length. Near the medio-dorsal abdominal line there are three pairs of spines, one pair situated about 0.19 mm. anterior to the vasiform orifice and the others on the cephalic part of the abdominal region. The spines of the pair on the first abdominal segment are somewhat more widely separated than those of the other two pairs. Six other pairs of spines are present on the abdomen. Five of these pairs are short, about 0.08 mm. long, and form an even subdorsal row on each side, the rows thus formed diverging on the cephalic part of the abdomen. The remaining pair is composed of much longer spines, situated about 0.29 mm. from the thoracic suture and about the same distance from the lateral margin of the case. On the thorax there is a subdorsal row of four spines on each side (fig. 1, *M*) and near the medio-dorsal line another pair of spines is present. Just anterior to the vasiform orifice a pair of tubercled setæ is situated, and another pair is present on the medio-caudal portion of case. The margin of the case (fig. 1, *F*) is dentate, the teeth (fig. 1, *K*, *L*) being rather fine and acute. A distance of 0.16 mm. is occupied by twelve of the teeth. At the base of the teeth small clear areas are found, and some distance in from the margin a row of elliptical areas, possibly glands, are present. These appear to be on the under surface of the case, while on the submarginal dorsal region, scattered between the margin and the insertion of the spines, are small dark pores. The vasiform orifice is situated on a tubercle which forms the caudal portion of the medio-dorsal ridge. It is subcircular in outline, tending to cordate. The operculum is somewhat similar in shape and obscures the lingula. The color of cleared specimens under the microscope is a light brown, with the margin and the borders of the dorsal ridge darker.

On the leaf the cases are shining black. There is little or no dorsal secretion, but a short, white, waxy marginal fringe is present. The rods forming this fringe are not distinct, but are more or less frayed and give a woolly appearance to the outer edge of the fringe. In some specimens, however, this woolly appearance is not evident, but the wax forms a series of marginal plates. When the pupæ are removed from the leaf, their former position is marked by the white oval wax ring which remains attached to the leaf. The larvæ present a similar appearance on the leaf, but are brown instead of black.

ADULT MALE.—Length 0.96 mm.; general color brownish, shaded with dusky. Vertex rounded, with a longitudinal median ridge, color dark brown; ocelli clear; compound eyes Vandyke, constricted; antennæ absent in the specimens at hand; labium tipped with dusky; thorax shaded with dusky. Forewings 0.88 mm. long by 0.35 mm. wide, marked with dark bluish gray, as indicated in fig. 1, *H*. Veins olive color; radial sector bent caudad at 0.4 mm. from the distal end. Hind wings 0.64 mm. long and 0.25 mm. wide at widest part; color uniform dusky, vein olive color. Legs with the femora and the proximal half of the tibiæ dusky, the remainder of the tibiæ and the tarsi greenish yellow. Fore femora 0.19 mm.; fore tibiæ 0.23 mm.; fore tarsi, proximal segment 0.08 mm., distal 0.064 mm.; middle femora 0.24 mm.,

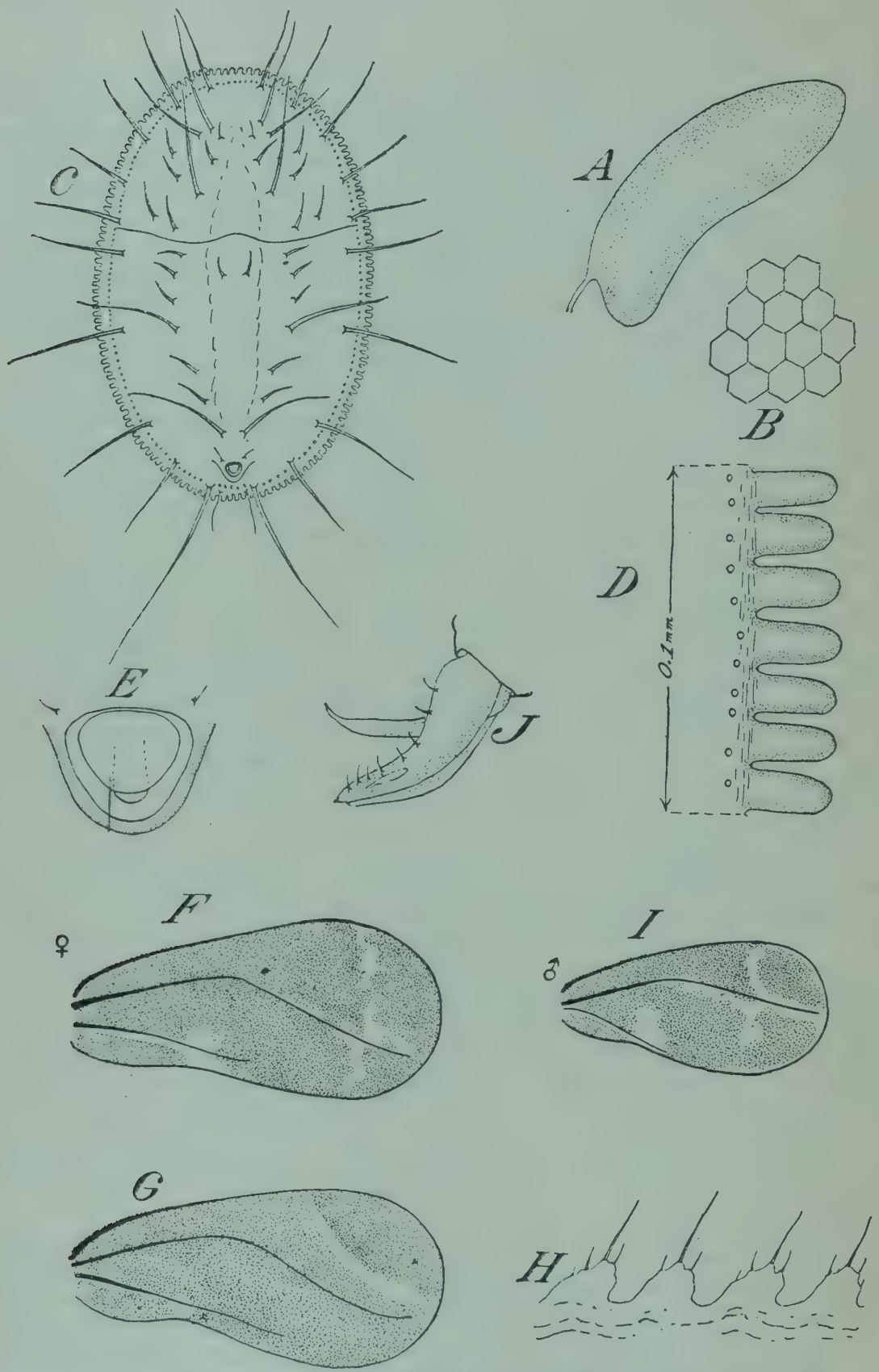


FIG. 2.—*Aleurocanthus woglumi*: *A*, egg; *B*, polygonal markings of egg; *C*, pupa case; *D*, margin of pupa case; *E*, vasiform orifice of pupa case; *F*, forewing of adult female; *G*, same, showing variation in markings; *H*, costal margin at base of wing of female; *I*, forewing of male; *J*, male genitalia.

hind tibiæ 0.36 mm., hind tarsus, proximal 0.112 mm., distal 0.72 mm. Claws normal, with a hairy central paronychium; genital segment dark brown, 0.112 mm., broad at the insertion of the claspers. These latter are dark-brown, becoming lighter at their distal tips. They are 0.128 mm. long and each about 0.03 mm. at the shoulder near the base. They are acute at the tips, curved inward, and armed on the inner margin with a number of prominent spines (fig. 1, *G*). A few small hairs are scattered here and there, situated on small tubercles. The penis is as long as the claspers, somewhat bulbous at the base, greenish yellow, and slightly curved upward.

ADULT FEMALE.—Unknown.

Described from adult males in balsam mounts and numerous pupa cases in balsam mounts and dry upon the foliage.

Type.—Cat. No. 19099, U. S. National Museum.

**Aleurocanthus woglumi** Ashby.[1]

*Aleurocanthus woglumi* Quain., Ashby, 1915, *in* Ann. Rpt. Dept. Agr. Jamaica, 1914/15, p. 31.
*Aleurocanthus woglumi* Quain., Ashby, 1915, *in* Bul. Dept. Agr. Jamaica, n. s. v. 2, no. 8, p. 322.

Specimens of this species (fig. 2; Pl. LXIV, LXV), which may be called the "spiny Citrus white fly," were first received by the Bureau of Entomology on June 16, 1910, from Dr. E. W. Berger, the material coming from India from H. Maxwell-Lefroy. Specimens were also received in 1910 from Mr. George Compere, who had collected the insect in the Philippine Islands. During 1910 and 1911 Mr. R. S. Woglum, in the course of his search for parasites of the orange white fly (*Dialeurodes citri* Ashm.), found this insect common and widely distributed on orange in India and Ceylon, and it has subsequently been received from that region from Mr. A. Rutherford.

Our first knowledge of its presence in the Western Hemisphere came with the receipt of specimens from Col. C. Kitchener, Half Way (Kingston), Jamaica, on November 27, 1913. Additional material was received during 1914 from Jamaica from Col. Kitchener and from Prof. S. F. Ashby, Microbiologist of the Jamaica Department of Agriculture. Under date of February 5, 1916, specimens were submitted by Mr. P. Cardin, Entomologist of the Cuba Agricultural Experiment Station, for verification of determination made by Prof. Ashby. On February 7, 1916, a large lot of orange leaves infested with *A. woglumi* was received from Mr. L. J. K. Brace, Nassau, New Province, Bahamas, who states:

> Certain orchards in this island at least have been very much affected with this insect, all of the leaves being so much infested on their undersurfaces that they present a black appearance, not only killing the trees but causing some persons to attempt to stop the mischief by cutting down the trees, though the young shoots become again covered * * *. I have no doubt that the planters' exchange have introduced this pest from the East. Plants have been for some time obtained by individuals here from the Jamaican establishment and also from Florida.

Prof. Ashby thinks the insect was introduced into Jamaica on the mango during the last 20 years. In that island it has become very

[1] *Aleurocanthus woglumi*, the writers' manuscript name for this species, was furnished to Prof. Ashby. According to the International Code, his descriptive remarks, as cited, make him the author of the species.

prominent, infesting the leaves of all species of Citrus on the lowland plains. Honeydew is excreted in small amounts, which is followed by the development of sooty fungi, but not to the extent that is true of certain other white flies and scale insects.

The present known distribution and food plants are shown in Table I.

TABLE I.—*Present known distribution and food plants of Aleurocanthus woglumi*

| Date. | Quaintance No. | Locality. | Host plant. | Collector. |
|---|---|---|---|---|
| June 16, 1910. | 5264 | India | Orange | Maxwell-Lefroy. |
| 1910 | 6763 | Manila, P. I. | do | George Compere. |
| 1910 | 6764 | do | do | Do. |
| Oct., 1910 | 6750 | Royal Bot. Gardens, Ceylon. | *Capparis roxburghi* | R. S. Woglum. |
| Do | 6744 | do | *Capparis pedunculosus* | Do. |
| (?) | 6553 | India | Unknown tree | Do. |
| Nov., 1910 | 6556 | Lahore, India | *Citrus* sp. | Do. |
| Do | 6564 | Gujranwala, India | do | Do. |
| Do | 6557 | Lahore, India | do | Do. |
| Do | 6560 | do | do | Do. |
| Dec., 1910 | 6528 | Kalimpong, Sikkim, India. | do | Do. |
| June, 1911 | 8021 | Lahore, India | *Citrus* sp. and *Morus* sp. | Do. |
| Sept., 1911 | 8012 | Nagpur, C. P., India | (?) | Do. |
| Aug., 1913 | 8753 | Peradeniya, Ceylon | *Salacia reticulata* | A. Rutherford. |
| Sept., 1913 | 8768 | do | *Kurrimia zeylanica* | Do. |
| Nov., 1913 | 8748 | Half Way, Jamaica | Orange | Col. C. Kitchener. |
| Feb., 1914 | 8782 | do | do | Do. |
| May, 1914 | 8922 | Kingston, Jamaica | *Citrus* sp.; *Guiacum officinale; Cestrum nocturnum* L. | S. F. Ashby. |
| Feb., 1916 | 12066 | Guantanamo, Cuba | Orange | P. Cardin. |
| Do | 12067 | Nassau, N. P., Bahama | do | L. J. K. Brace. |

EGG (fig. 2, *A*).—Size, 0.208 mm. by 0.08 mm.; shape elliptical, curved, with the stalk short and attached some distance from the base. Color yellowish, surface apparently without reticulations in some cases and with them in others, which is no doubt due to the structure being destroyed in boiling. When they are present (fig. 2, *B*) they average 0.006 mm. in diameter.

LARVA.—Larvæ are present in the material at hand, but they are in too poor a condition for accurate description. They are brown in color and armed with numerous long spines.

PUPA CASE (fig. 2, *C*).—Size variable in the different lots of material, averaging 1.4 by 0.89 mm.; shape regularly elliptical, with the dorsum considerably arched or rounded; the median ridge high, but not markedly distinct from the dorsal area, excepting near the caudal portion of the abdomen and at the vasiform orifice, which is elevated into a more or less prominent tubercle. Color dense black, so much so that it is almost impossible, even after prolonged boiling, to make out details. When the denser dorsal portion of the case is removed the ventral part appears under the microscope as dark brown and more or less irregularly mottled. Submarginal area with usually 20 spines forming a ring. These vary consideably in length, but the caudal pair is nearly always the longest. The spines are curved outward. A pair of hairs is present on the caudal margin caudad of the vasiform orifice. The spines on the dorsum are small excepting two pairs on the abdomen and three pairs on the thorax. Their number and arrangement are shown in the figure. The vasiform orifice (fig. 2, *E*) is prominent, being on a tubercle, but is small. It is somewhat triangular in shape, tending to circular. The operculum almost entirely fills the orifice obscuring the lingula—all but a very small portion at the tip. Cephalad of the orifice a pair of minute setæ is situated one on each side. The margin of the case

is dentate, the teeth large and bluntly rounded (fig. 2, *D*). The inner spaces are not acute, but often squarely truncate. A space of 0.1 mm. is occupied by six or seven teeth. On this feature alone the case is easily separable from those of the other species. At the base of the teeth, forming a ring around the case, is a series of minute, clear, porelike areas. On the leaf the case is jet black with the dorsum somewhat arched and the abdominal segments marked, but not distinctly separated. On the margin all around is a narrow cottony lateral wax fringe. This sometimes extends mesad, irregularly covering the submarginal area, but dorsal secretion is usually absent.

ADULT FEMALE.—Length from vertex to tip of ovipositor, 1.12 mm.; color brown, under the microscope a deep wine color with darker shadings on head, thorax, and tip of abdomen. The specimens at hand are somewhat imperfect and it is difficult to make out the structure. The vertex seems to be rounded and possessed of a slight median ridge. The eyes are very dark brown. The antennæ are absent from the specimens at hand. Labium yellowish, tipped with black. Legs yellowish, shaded on femora with dusky. The femora and tibiæ of the hind legs are considerably darker than the others; length of hind femora 0.288 mm.; hind tibiæ 0.432 mm. The tarsi have the proximal segment 0.1 mm. and the distal 0.06 mm. The proximal segment is armed on its distal extremity with one large spine and several smaller ones; the foot is normal, with the paronychium straight and hairy. The forewings (figs. 2, *F*, *G*) are 1.268 mm. long and 0.76 mm. wide at the widest part. The radial sector is heavy, yellowish brown in color, and much curved. The cubitus is very fine, long and slightly curved, that portion of the wing below it forming a more or less distinct lobe. In color the wing is a deep smoky, excepting as follows: A line following the cubitus, and a rather large spot near its distal extremity are colorless. A line following the radial sector from its distal extremity to almost its median curve, and another crossing it almost at right angles are colorless. This gives the appearance of a white cross on a dark background. In some wings the marking is not so evident, but there is one curved colorless line angling across the wing a short distance above and parallel with the radial sector. The border of this white line seems more heavily shaded than the remainder of the wing. The margin of the wing (fig. 2, *H*) is armed with a series of rather prominent teeth directed toward the distal extremity of the wing. Each one of these is armed with one prominent spine and usually three smaller ones. The margin formed by these teeth and a line along their bases is bright wine red. The hind wing is uniform smoky, with the vein yellowish brown.

ADULT MALE.—Much smaller than the female, measuring only about 0.79 mm. from vertex to tip of claspers. The specimens are in poor condition, the antennæ are absent, and it is impossible to make out the structure with certainty. The color is a yellowish or a reddish brown. The hind femora, 0.24 mm. and the hind tibia, 0.4 mm. in length. They are marked as in the female. The claspers (fig. 2, *J*) are 0.126 mm. long. Near their distal ends there are a number of jagged teeth and they are armed with a number of long slightly curved hairs, those near the tip being the longest. The penis is as long as the claspers, yellowish, and almost straight.

Described from females, males, and pupa cases in balsam mounts and pupa cases and eggs on the leaves.

**Aleurocanthus spiniferus** (Quaintance)

*Aleurodes spinifera* Quain., 1903, *in* Canad. Ent., v. 35, no. 3, p. 63.

Collected on *Citrus* sp. and rose by Mr. C. L. Marlatt, of the Bureau of Entomology, at Garalt, Java, on December 7, 1901; also taken on orange at Macao, South China, by Mr. R. S. Woglum, in February, 1911.

**Aleurolobus marlatti** (Quaintance)

*Aleurodes marlatti* Quain., 1903, in Canad. Ent., v. 35, no. 3, p. 61.

This species (Pl. LXVI, fig. 3) was collected by Mr. C. L. Marlatt on May 17, 1901, at Kumomoto, Japan, on orange; also by Mr. R. S. Woglum on *Citrus* sp. and *Morus* sp. at Lahore, India; also collected by Mr. Woglum on *Ficus* sp. in the Royal Botanic Gardens, Ceylon; on an unknown tree in the Botanic Gardens, Buitenzorg, Java. This insect has also been received by the Bureau of Entomology from Mr. S. I. Kuwana, collected at Fukuoka, Japan. Mr. Kuwana states that this same species has been collected on Rivkin Island. One lot of infested orange leaves is also in the Bureau collection from Tokyo, Japan.

**Aleurothrixus floccosus** (Maskell)

*Aleurodes floccosa* Mask., 1896, *in* Trans. and Proc. N. Zeal. Inst., v. 28 (n. s. v. 11), 1895, p. 432.
*Aleurodes horridus* Hempel, 1899, *in* Psyche, v. 8, no. 280, p. 394.

This species (fig. 3, *H*) is based on material from Jamaica on lignum-vitæ (*Guaiacum officinale*?) and was first recorded on orange by Cockerell (1902) [1] from Mexico. The insect has several color phases, ranging from clear yellow, the typical and more abundant form, to individuals with the dorsum striped with dark brown, or with the dorsal disk dark brown and the submarginal area yellow, etc.

Hempel's *A. horridus* from Brazil on guava (*Psidium guajava*) is apparently the same as *A. floccosus*. This latter differs from *A. howardi* only in the absence of a comb of teeth on the caudal margin of the vasiform orifice (fig. 3, *H*). Both *A. floccosus* and *A. howardi* are almost always present together on the same leaf and their food plants and distribution are practically identical. *A. floccosus* is common in the islands of the West Indies and also occurs in Florida, Mexico, British Guiana, Brazil, Argentina, Canal Zone, Chile, and Paraguay. In addition to the orange, lime, grapefruit, etc., *A. floccosus* has been taken on the sea-grape (*Coccoloba uvifera*), *Plumeria* sp., *Baccharis genistelloides*, guava, a coarse grass, and a climbing vine.

**Aleurothrixus howardi** (Quaintance)

*Aleyrodes howardi* Quain., 1907, U. S. Dept. Agr. Bur. Ent. [Bul.] 12, pt. 5, Tech. Ser., p. 91.

This species (fig. 3, *E*, *J*; Pl. LXVII) occurs on the same host plant and has the same distribution as *A. floccosus*. It was apparently first found in Florida by Prof. P. H. Rolfs at Miami on sea-grape, September 25, 1900, and therefore gained a foothold in that State some years previous to its discovery by Dr. E. A. Back.

**Aleurothrixus porteri**, n. sp.

This species (fig. 3, *A–D*, *F*, *G*, *I*, *K*, *L*; Pl. LXVIII) has been received only from Chile and Brazil. The first collection was sent by Prof. T. D. A.

[1] Bibliographic citations in parentheses refer to "Literature cited," pp. 471–472.

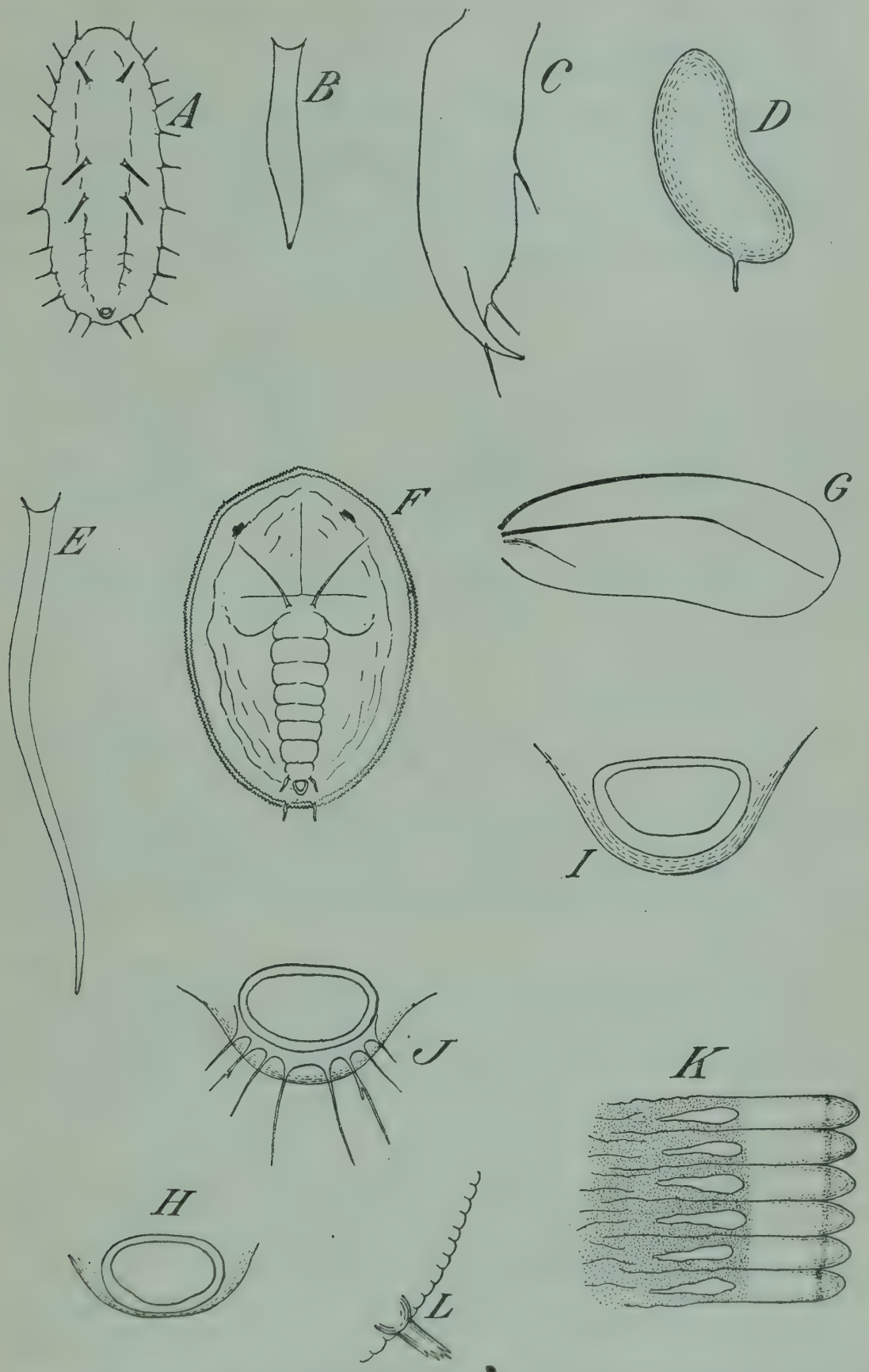


FIG. 3.—*Aleurothrixus porteri, A. howardi,* and *A. floccosus: A, Aleurothrixus porteri:* Larva, first instar. *B, A. porteri:* Caudal spine of pupa case. *C, A. porteri:* Clasper of male. *D, A. porteri:* Egg. *E, A. howardi:* Caudal spine. *F, A. porteri:* Pupa case. *G, A. porteri:* Forewing of adult. *H, A. floccosus:* Vasiform orifice of pupa case. *I, A. porteri:* Vasiform orifice of pupa case. *J, A. howardi:* Vasiform orifice of pupa case. *K, A. porteri:* Margin of pupa case. *L, A. porteri:* Margin of early larva.

Cockerell on June 7, 1895, who received the material from Mr. Lataste, under the name *phalaenoides*.

In a letter to the senior author in January, 1905, Cockerell suggested that Lataste supposed the species to be Blanchard's *phalaenoides*. Since that time we have shown that *phalaenoides* Blanchard is a species of Aleurodicus. Table II records the distribution and food plants of the specimens of *A. porteri* in the collection of the Bureau of Entomology.

TABLE II.—*Distribution and food plants of Aleurothrixus porteri in the collection of the Bureau of Entomology*

| Date. | Collector. | Host. | Bureau No. | Locality. |
|---|---|---|---|---|
| Feb. 13, 1894.. | M. Lataste..... | Orange..... | Q. 4062.... | Santiago, Chile. |
| May 14, 1894.. | .....do..... | .....do..... | Q. 4063.... | Do. |
| Mar. 14, 1895.. | .....do..... | (?)..... | Q. 4064.... | Chile. |
| Feb. 4, 1896... | Edward Reed..... | Orange..... | Q. 4065.... | Ransagua, Chile. |
| Apr. 1, 1899... | D. G. Fairchild..... | Solanaceous plant..... | Q. 351..... | Villa del Mar, Chile. |
| Oct., 1904.... | M. J. Rivera..... | Orange?..... | Q. 3214.... | San Bernardo, Chile. |
| Oct. 25, 1904.. | .....do..... | *Schinus dependens* Ortega. | Q. 12022... | Santiago, Chile. |
| June 20, 1912. | Prof. Carlos E. Porter.. | *Schinus molle*..... | Q. 8726.... | Chile. |
| Mar., 1913.... | .....do..... | Orange..... | Q. 8820.... | Santiago, Chile. |
| Jan. 5, 1914... | Popenoe and Dorsett.... | Jaboticaba..... | Q. 12004... | Rio de Janeiro, Brazil. |
| (?)..... | Prof. Carlos E. Porter... | *Lippia citriodora* Kunth. | Q. 12024... | Santiago, Chile. |
| Aug. 8, 1915.. | .....do..... | Myrtus..... | Q. 12062... | Do. |

Of this material, Quaintance No. 351 is chosen for the type.

LARVA, FIRST STAGE (fig. 3, *A*).—Size 0.352 by 0.208 mm. Shape elongate elliptical; abdomen with a moderately distinct keel, the caudal extremity of which projects to the vasiform orifice; dorsum armed with four pairs of stout straight spines; margin very minutely serrate and armed on its caudal part with a pair of long curved spines and the remainder of the margin with 11 pairs of minute spines; antennæ straight, not quite as thick as the dorsal spines and extending slightly beyond the margin; vasiform orifice almost completely filled by the operculum; color under the microscope pale brown.

PUPA CASE.—Size 0.88 by 0.502 mm.; shape elliptic, some specimens slightly broader across the thorax than across the abdomen; dorsum somewhat elevated, the abdomen with a distinct keel; incisions between marginal wax tubes shallow; vasiform orifice (fig. 3, *I*) small, elevated, operculum filling about half of the orifice and obscuring the lingula; spines latero-cephalad of the vasiform orifice and those on the caudal margin of case short, stout, and somewhat vasiform (fig. 3, *B*); those on the medio dorsum long; other characters very similar to those of *A. floccosus*. Color varying from yellow to dark brown and with flocculent wax as in *A. floccosus*.

ADULT MALE.—Color yellow, eyes dark brown; clasper rather short (fig. 3, *C*), its spur acute and not armed within with lobes; a few prominent spines present; length 0.08 mm.; length of insect from vertex to tip of claspers 0.88 mm.; forewing 1.04 mm. long, without markings, but often uniformly clouded with dusky.

ADULT FEMALE.—Similar to male in color; length 1.12 mm.; forewing 1.28 mm.

The adults in the collection are poorly preserved, and it is impossible to describe them in detail.

Described from larvæ, pupa cases, and adults in balsam mounts and pupa cases upon foliage.

Type.—Cat. No. 20171, U. S. National Museum.

**Bemisia giffardi** (Kotinsky)

*Aleyrodes giffardi* Kotin., 1907, *in* Bd. Com. Agr. and Forest. Hawaii Div. Ent. Bul. 2, p. 94.

This insect is reported present on Citrus trees in several gardens in Honolulu, where it is stated to be so abundant that the foliage of the trees becomes blackened by the sooty fungus growing on the exuded honeydew. Mr. Kotinsky believes that the insect has been introduced into Hawaii, and this opinion is strengthened by its discovery in collections of material made by Mr. Woglum at Lahore, India, in 1911. The host, however, was an unknown tree.

**Dialeurodes citri** (Ashmead) [1]

*Aleyrodes citri* Riley and Howard, 1893, *in* Insect Life, v. 5, no. 4, p. 219.
*Aleurodes eugeniae*, var. *aurantii* Mask., 1896, *in* Trans. and Proc. N. Zeal. Inst., v. 28 (n. s. v. 11), 1895, p. 431.
*Aleyrodes aurantii* Ckll., 1903, *in* Fla. Agr. Exp. Sta. Bul. 67, p. 665.

This is the destructive Citrus white fly of Florida, where it has been known since about 1880 (Pl. LXVI, fig. 1). It is rather generally distributed over the orange-growing regions of the Gulf States and is common on chinaberry and Cape jasmine considerably north of the Citrus belt. It is also recorded from Colorado, Illinois, and the District of Columbia, where it is probably confined to conservatories. This insect was discovered in California in 1907 and serious attempts were made to effect its eradication. It is still present in one locality (Marysville), where it is now so widespread and abundant that its eradication is considered to be impracticable (Weldon, 1915).

*Dialeurodes citri* is undoubtedly of oriental origin. It has been received from numerous localities in India, Ceylon, Japan, China, etc. According to Kirkaldy it is present in Chile, Mexico, and Brazil. In addition to Citrus plants, the insect in Florida infests numerous others as *Melia azederach*, *Gardenia jasminoides*, *Ligustrum* spp., *Diospyros kaki*, *Diospyros virginiana*, *Syringa* sp., *Coffea arabica*, *Ficus nitida*, etc. This and nearly related species are very generally parasitized in the Orient by certain four-winged flies, which are in that region apparently effective checks on their undue increase.

**Dialeurodes citrifolii** (Morgan)

*Aleyrodes citrifolii* Morgan, 1893, La. Agr. Exp. Sta. Spec. Bul., p. 70.
*Aleyrodes nubifera* Berger, 1909, Fla. Agr. Exp. Sta. Bul. 97, p. 67.
*Aleyrodes nubifera* Mor. and Back, 1911, U. S. Dept. Agr. Bur. Ent. Bul. 92, p. 86.

This species, long confused with *D. citri*, may be readily distinguished from that species by the reticulate eggs, character of the tracheal folds of the pupa case, and the smoky patch on front wings of the adults. The insect is known from North Carolina, Mississippi, Louisiana, California,

[1] This species was first fully described by Riley and Howard in Insect Life, as cited, but had earlier been named and briefly described in The Florida Dispatch, November, 1885, by W. H. Ashmead, who, according to the rules of the International Code, must be known as the author of the species.

and Florida. While not as important as *D. citri*, it is nevertheless decidedly noxious. It is also known to occur in Cuba and Mexico. No specimens of this insect were found in the Woglum collection of white flies from India, Ceylon, and other points in the East visited by him. By reason of its affinities, *D. citrifolii* is, however, almost surely oriental in origin.

This species, with one exception, is known to attack only Citrus plants. It was found on *Ficus nitida* growing in greenhouses at Audubon Park, New Orleans, La.

**Paraleyrodes perseae** (Quaintance)

*Aleurodes perseae* Quain., 1900, U. S. Dept. Agr. Div. Ent. [Bul.] 8, Tech. Ser., p. 32.
*Paraleyrodes perseae* Quain. and Baker, 1913, U. S. Dept. Agr. Bur. Ent. [Bul.] 27, pt. 1, Tech. Ser., p. 82,

This species is known only from Florida, where it is frequently found on orange, though never in destructive numbers thus far. It also feeds upon *Persea*, the avocado (*Persea americana*), and doubtfully on persimmon (*Diospyros* spp.). Several species of the genus are common in the West Indies, *perseae* being the only one known from the United States.

**Trialeurodes floridensis** (Quaintance)

*Aleurodes floridensis* Quain., 1900, U. S. Dept. Agr. Bur. Ent. [Bul.] 8, Tech. Ser., p. 26.

*T. floridensis* has thus far been recorded by the Bureau of Entomology only from Florida, where it is rather generally distributed. It infests avocado, guava, *Annona squamosa*, and the orange. While often very numerous on guava and avocado, it is at present of no importance on orange.

**Trialeurodes vitrinellus** (Cockerell)

*Aleyrodes vitrinellus* Ckll., 1903, *in* Ent. News, v. 14, no. 7, p. 241.

The type of this species is from Mexico on orange. Apparently the same insect has been taken in southern California on oak. Its injuries to orange in Mexico are probably not great.

**Tetraleurodes mori** (Quaintance)

*Aleurodes mori* Quain., 1899, *in* Canad. Ent., v. 31, no. 1, p. 1.

This indigenous species (Pl. LXIX, fig. 2) is widely distributed over the eastern United States and occurs on a large variety of plants, as mulberry, sycamore, maple, dogwood, hackberry, persimmon, holly, mountain laurel, etc. It has been found several times on orange, but not as yet in injurious numbers. That it may become troublesome under certain conditions, however, will be evident from the discussion relative to *T. mori*, var. *arizonensis*, which follows:

**Tetraleurodes mori,** var. **arizonensis** (Cockerell)

*Aleyrodes mori,* var. *arizonensis* Ckll., 1903, *in* Fla. Agr. Exp. Sta. Bul. 67, p. 666.
*Aleurodes mori* Ckll., 1900, *in* Sci. Gossip, n. s. v. 6, no. 72, p. 366.

Described from specimens taken in Arizona on orange (Pl. LXIX). The variety *T. mori arizonensis* is stated to differ from the typical *T. mori* in having the wings white marked with black without any red. An examination of the type specimens after mounting shows the presence of red markings on wings exactly as in *T. mori,* and we are unable to distinguish any characters in support of its status as a variety. On different occasions the Bureau of Entomology has received from Mexico an aleyrodid seriously infesting the orange (Pl. LXIX) which we are unable to distinguish in the immature stages from *T. mori,* and this species is considered by Cockerell to be identical with his variety *T. mori arizonensis.* While the variety, in our judgment, is invalid, we retain the name to designate a race of *T. mori* which, in Mexico, for some reason breeds abundantly on orange and is a pest of importance. *T. mori arizonensis* occurs only on orange in Mexico so far as bureau records indicate. It was first collected in 1894 by Dr. C. H. T. Townsend at Guadalajara and San Luis, and again by Townsend in 1902 at Zapotlan. Two lots of material were received from Prof. A. L. Herrera in 1905, without statement as to locality.

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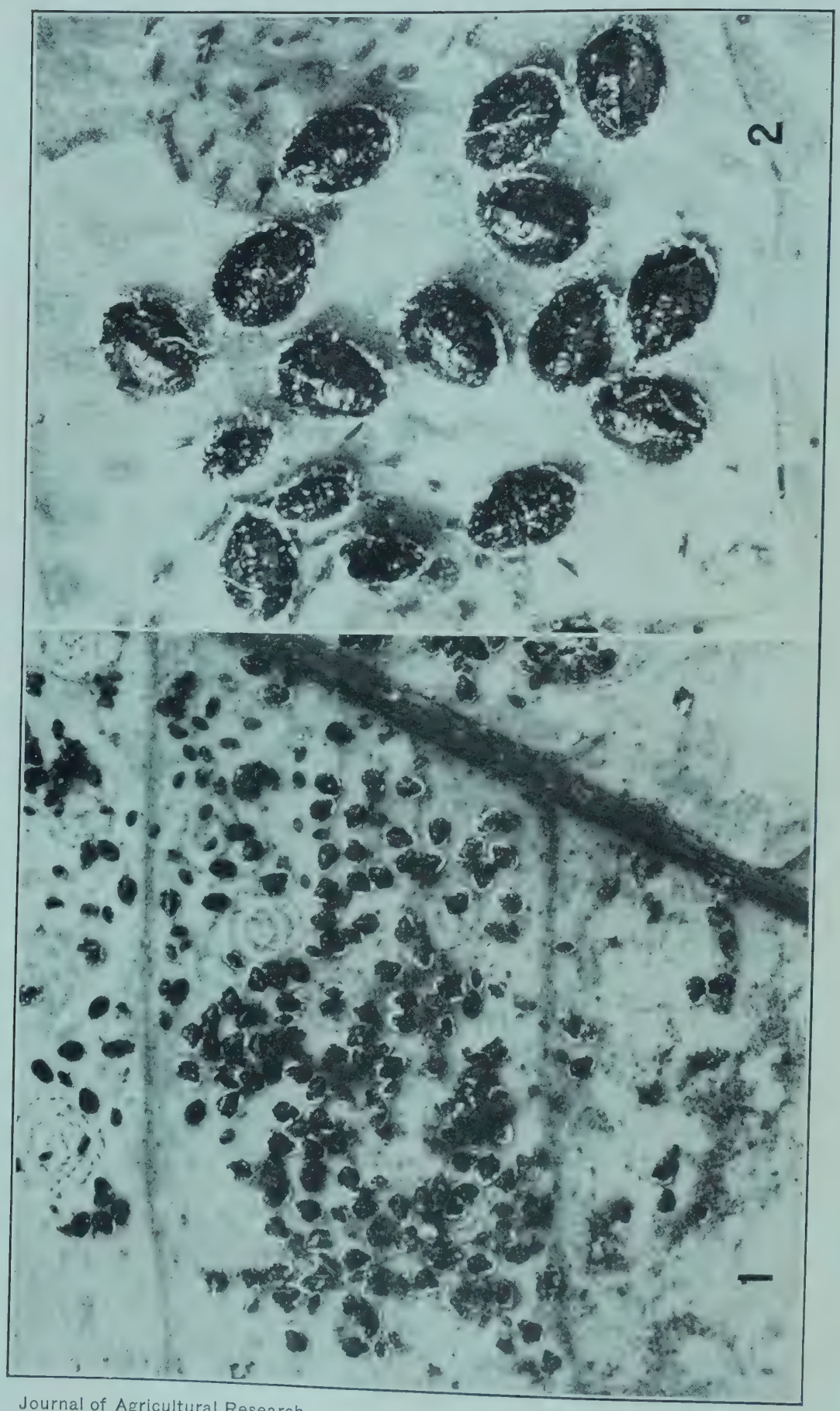
## PLATE LXIV

*Aleurocanthus woglumi:* Eggs, larvæ, and pupa cases on orange leaves.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## PLATE LXV

*Aleurocanthus woglumi:*

Fig. 1.—Colony on an orange leaf.
Fig. 2.—Eggs and pupa cases, greatly enlarged

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## PLATE LXVI

Fig. 1.—*Dialeurodes citri:* Pupæ, much enlarged.
Fig. 2.—Male and female adults of an aleyrodid.
Fig. 3.—*Aleurolobus marlatti,* much enlarged.

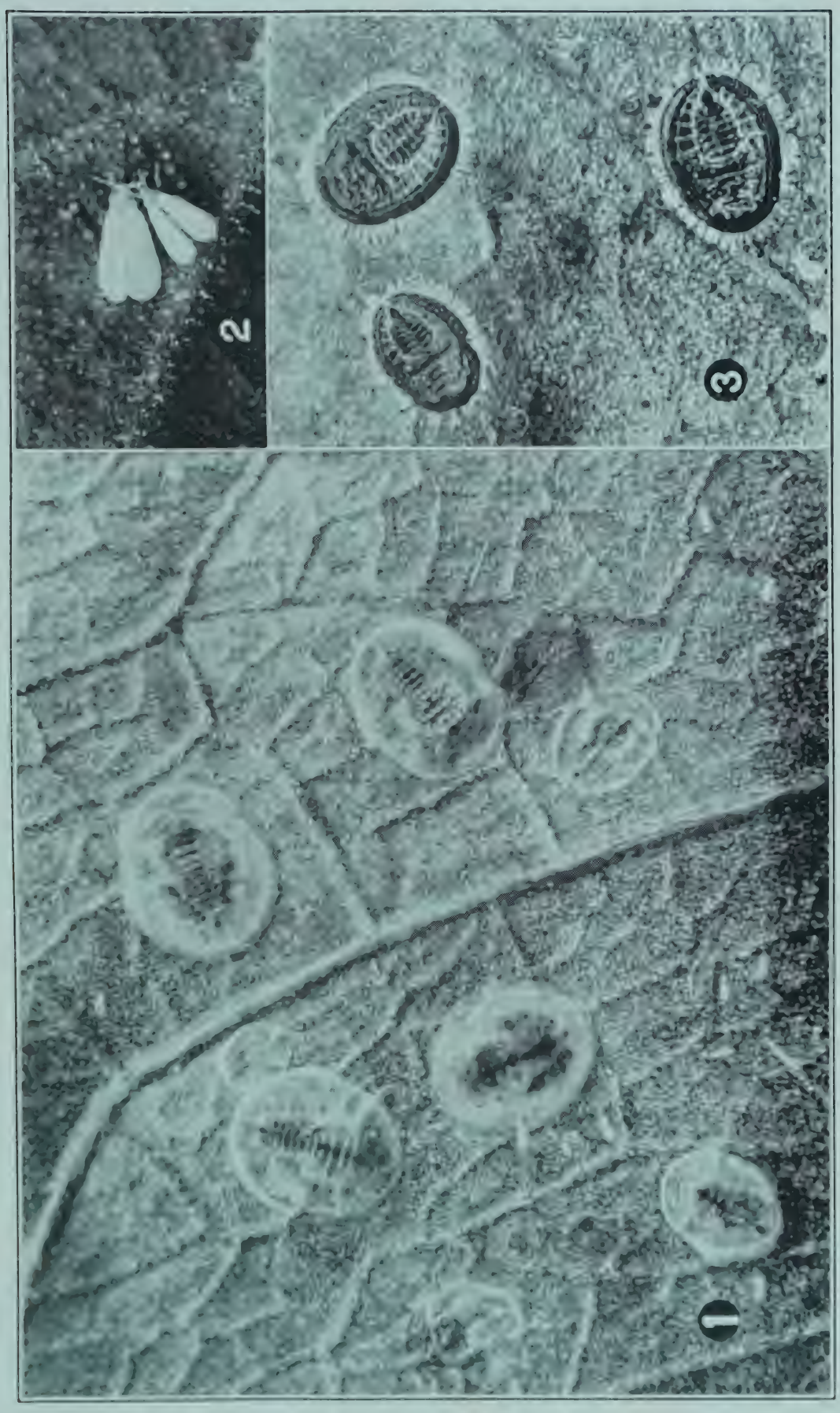
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PLATE LXVII

## PLATE LXVII

*Aleurothrixus howardi:* Larvæ and pupa cases on an orange leaf, enlarged.

## PLATE LXVIII

*Aleurothrixus porteri:* Larvæ and pupa cases on *Myrtus* sp., enlarged.

1
2

## PLATE LXIX

Fig. 1.—*Tetraleurodes mori*, var *arizonensis:* Larvæ and pupa cases on an orange leaf, enlarged.

Fig. 2.—*Tetraleurodes mori:* Pupa cases on a mulberry leaf, much enlarged.

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# RELATIVE WATER REQUIREMENT OF CORN AND THE SORGHUMS

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## INTRODUCTION

During the summers of 1914 and 1915 a physiological study of the water relations of corn and the nonsaccharin sorghums was made at the State Branch Experiment Station at Garden City, Kans. In connection with other experiments it was thought advisable to determine the water requirement of several varieties of these plants. The term "water requirement," as used in this paper, means the ratio of the weight of the water absorbed by the plant to the weight of the dry matter produced.

## EXPERIMENTAL METHODS

### CLIMATIC DATA

The instruments for recording the climatic conditions consisted of a hydrograph, a thermograph, maximum and minimum thermometers placed in a standard shelter 4 feet from the ground, a rain gauge, an evaporation tank, and an anemometer which measured the wind velocity at a height of 2 feet.

A portion of the weather records for the two seasons averaged for five-day periods is shown in Table I. These show that the climatic conditions for the two seasons were in marked contrast. The summer of 1915 was much cooler than that of 1914 and the rainfall for the months of May, June, July, August, and September in 1915 was approximately three times that for the same months in 1914. The evaporation during 5-day periods is shown graphically in figure 1.

The evaporation for each of the growing months with but one exception was much higher in 1914 than in 1915.

### CULTURAL METHODS

The plants were grown in large metal cans made from 22-gauge galvanized iron. These cans were 24 inches in height with a diameter of 15 inches, and under the conditions of these experiments contained about

110 kgm. of soil. Forty of these cans were used in 1914 and 60 in 1915. The upper foot of field soil was worked through a sieve with a ¼-inch mesh and then thoroughly tamped in the cans. The soil was in good tilth, and for both seasons the moisture content ranged from 20 to 21 per cent (dry basis). It had a moisture equivalent of 24 and a wilting coefficient of 13, as calculated by the formula of Briggs and Shantz.[1]

The cans were provided with metal lids which were sealed with ordinary binding tape (Pl. LXXII, fig. 4). This was made waterproof by

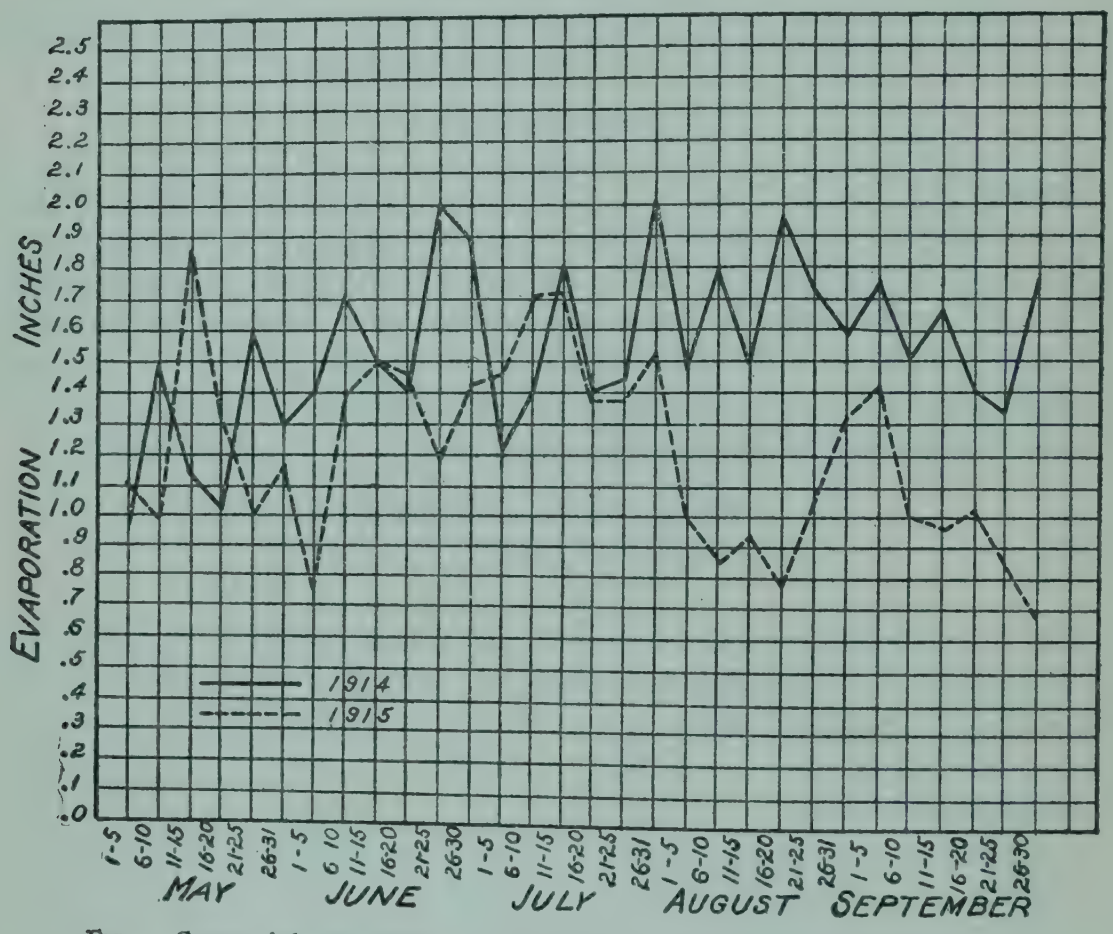



FIG. 1.—Curves of the evaporation at Garden City, Kans., for the growing period of 1915.

giving it a heavy coat of varnish after it was in position. Three 2-inch holes equidistant from one another were made near the periphery of each lid to accomodate the plants. The seeds were planted in the cans and the young plants gradually thinned to the desired number. Three corn plants were grown in each can, both in 1914 and 1915. Six sorghum plants were grown in each can in 1914, but in 1915 the number was reduced to three plants to each can. In order that the plants might be as nearly as possible under the same climatic conditions during the growing season, the seeds of all the plants used were sowed on the same date. These were planted on May 26 in 1914, and on May 22 in 1915.

[1] Briggs, L. J., and Shantz, H. L. The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agr. Bur. Plant Indus. Bul. 230, 83 p., 9 fig., 2 pl. 1912.

TABLE I.—*Summary of the climatic conditions at Garden City, Kans., for 1914 and 1915*

| Period (inclusive). | Air temperature (°F.). | | | | | Precipitation. | Evaporation. | Wind velocity per hour. |
|---|---|---|---|---|---|---|---|---|
| | Average of— | | | Maximum. | Minimum. | | | |
| | Means. | Maximums. | Minimums. | | | | | |
| 1914. | | | | | | | | |
| May: | | | | | | *Inches.* | *Inches.* | *Miles.* |
| 1 to 5 | 58 | 68 | 47 | 78 | 44 | 1.40 | 0.953 | 9.0 |
| 6 to 10 | 65 | 78 | 51 | 92 | 41 | .19 | 1.484 | 11.8 |
| 11 to 15 | 53 | 61 | 44 | 72 | 38 | .20 | 1.135 | 10.9 |
| 16 to 20 | 62 | 68 | 55 | 79 | 50 | .72 | .596 | 13.6 |
| 21 to 25 | 72 | 84 | 59 | 90 | 57 | .12 | 1.584 | 10.2 |
| 25 to 31 | 69 | 79 | 57 | 89 | 49 | 1.00 | 1.294 | 6.9 |
| June: | | | | | | | | |
| 1 to 5 | 76 | 87 | 65 | 92 | 62 | .19 | 1.432 | 13.0 |
| 6 to 10 | 77 | 89 | 64 | 91 | 51 | .21 | 1.728 | 15.2 |
| 11 to 15 | 76 | 88 | 63 | 96 | 59 | .61 | 1.520 | 9.3 |
| 16 to 20 | 76 | 89 | 62 | 99 | 58 | .39 | 1.409 | 6.4 |
| 21 to 25 | 82 | 94 | 69 | 98 | 64 | ..... | 1.991 | 9.9 |
| 26 to 30 | 77 | 94 | 59 | 103 | 51 | .04 | 1.862 | 7.7 |
| July: | | | | | | | | |
| 1 to 5 | 74 | 85 | 62 | 94 | 53 | .15 | 1.200 | 6.1 |
| 6 to 10 | 77 | 91 | 60 | 93 | 53 | .10 | 1.440 | 4.7 |
| 11 to 15 | 86 | 99 | 69 | 103 | 64 | ..... | 1.822 | 5.7 |
| 16 to 20 | 76 | 87 | 62 | 101 | 58 | .21 | 1.416 | 7.7 |
| 21 to 25 | 81 | 94 | 65 | 98 | 64 | .10 | 1.451 | 5.7 |
| 26 to 31 | 83 | 98 | 66 | 102 | 64 | Trace. | 2.074 | 5.7 |
| August: | | | | | | | | |
| 1 to 5 | 77 | 93 | 65 | 95 | 61 | .38 | 1.477 | 6.1 |
| 6 to 10 | 77 | 91 | 62 | 95 | 56 | Trace. | 1.792 | 8.0 |
| 11 to 15 | 77 | 91 | 62 | 95 | 58 | .19 | 1.474 | 7.0 |
| 16 to 20 | 82 | 99 | 64 | 102 | 62 | .06 | 1.959 | 8.2 |
| 21 to 25 | 77 | 91 | 61 | 99 | 50 | .01 | 1.745 | 7.5 |
| 25 to 31 | 73 | 87 | 60 | 94 | 54 | Trace. | 1.563 | 7.4 |
| September: | | | | | | | | |
| 1 to 5 | 77 | 94 | 60 | 103 | 55 | ..... | 1.739 | 7.5 |
| 6 to 10 | 79 | 96 | 64 | 102 | 59 | .01 | 1.501 | 8.6 |
| 11 to 15 | 75 | 89 | 58 | 96 | 48 | .03 | 1.653 | 11.4 |
| 16 to 20 | 77 | 90 | 60 | 97 | 56 | ..... | 1.390 | 7.6 |
| 21 to 25 | 63 | 80 | 44 | 85 | 37 | .11 | 1.343 | 6.4 |
| 26 to 30 | 67 | 86 | 51 | 90 | 47 | ..... | 1.740 | 11.1 |
| 1915. | | | | | | | | |
| May: | | | | | | | | |
| 1 to 5 | 53 | 65 | 38 | 76 | 31 | .79 | 1.187 | 10.0 |
| 6 to 10 | 56 | 69 | 44 | 81 | 32 | ..... | .985 | 7.7 |
| 11 to 15 | 71 | 87 | 55 | 94 | 46 | ..... | 1.857 | 10.8 |
| 16 to 20 | 46 | 55 | 39 | 68 | 32 | 2.38 | 1.324 | 12.2 |
| 20 to 25 | 67 | 78 | 57 | 90 | 44 | .07 | 1.069 | 8.6 |
| 25 to 31 | 55 | 65 | 47 | 72 | 39 | 1.15 | 1.169 | 8.1 |
| June: | | | | | | | | |
| 1 to 5 | 65 | 75 | 58 | 81 | 55 | .64 | .738 | 8.7 |
| 6 to 10 | 64 | 78 | 52 | 86 | 36 | .94 | 1.386 | 8.6 |
| 11 to 15 | 66 | 78 | 53 | 87 | 50 | ..... | 1.490 | 8.0 |
| 16 to 20 | 71 | 85 | 61 | 95 | 56 | .07 | 1.485 | 8.8 |
| 21 to 25 | 69 | 79 | 58 | 91 | 56 | .62 | 1.181 | 8.5 |
| 26 to 30 | 72 | 84 | 59 | 88 | 57 | .69 | 1.419 | 7.1 |
| July: | | | | | | | | |
| 1 to 5 | 66 | 77 | 55 | 83 | 49 | .57 | 1.451 | 8.8 |
| 6 to 10 | 76 | 90 | 60 | 96 | 54 | .51 | 1.732 | 8.6 |

TABLE I.—*Summary of the climatic conditions at Garden City, Kans., for 1914 and 1915*—Continued

| Period (inclusive). | Air temperature (°F). | | | | | Precipitation. | Evaporation. | Wind velocity per hour. |
|---|---|---|---|---|---|---|---|---|
| | Average of— | | | Maximum. | Minimum. | | | |
| | Means. | Maximums. | Minimums. | | | | | |
| 1915. | | | | | | | | |
| July—Continued. | | | | | | *Inches.* | *Inches.* | *Miles.* |
| 11 to 15 | 81 | 97 | 67 | 101 | 64 | 0.06 | 1.743 | 6.7 |
| 16 to 20 | 72 | 84 | 62 | 96 | 56 | .15 | 1.407 | 7.0 |
| 21 to 25 | 74 | 85 | 61 | 91 | 56 | .13 | 1.397 | 5.5 |
| 25 to 31 | 75 | 74 | 64 | 90 | 62 | .24 | 1.528 | 6.8 |
| August: | | | | | | | | |
| 1 to 5 | 69 | 83 | 56 | 90 | 51 | .90 | 1.012 | 5.8 |
| 6 to 10 | 70 | 80 | 60 | 94 | 56 | 5.11 | .860 | 4.9 |
| 11 to 15 | 72 | 83 | 61 | 86 | 59 | .10 | .927 | 2.7 |
| 16 to 20 | 61 | 80 | 61 | 84 | 57 | .03 | .790 | 3.2 |
| 21 to 25 | 70 | 81 | 60 | 84 | 57 | .46 | 1.018 | 4.4 |
| 25 to 31 | 63 | 77 | 50 | 85 | 40 | ..... | 1.313 | 4.7 |
| September: | | | | | | | | |
| 1 to 5 | 68 | 83 | 55 | 87 | 51 | .82 | 1.424 | 7.4 |
| 6 to 10 | 69 | 81 | 56 | 91 | 54 | ..... | 1.029 | 6.3 |
| 11 to 15 | 71 | 84 | 60 | 97 | 53 | Trace. | .983 | 7.2 |
| 16 to 20 | 69 | 82 | 55 | 87 | 39 | .20 | 1.072 | 5.2 |
| 21 to 25 | 66 | 76 | 58 | 84 | 50 | 1.00 | .864 | 18.2 |
| 25 to 30 | 56 | 67 | 48 | 78 | 44 | .25 | .665 | 4.4 |

The holes in the lids were made water-tight by using a mixture of approximately 16 parts by weight of beeswax to 1 part of Venetian turpentine. Under ordinary conditions the young seedlings of the corn and sorghum can readily penetrate this wax. After the plants had emerged through the wax, it was replaced by a mixture containing a much smaller amount of Venetian turpentine, in order to secure a seal that would remain firm around the plants during the hot summer weather. The lids of the cans were given a heavy coat of white paint and were then covered with a layer of burlap in order to protect them from excessive heat. The water lost by the plants was replaced every 48 hours by the method used by Briggs and Shantz [1] in their extensive work on the water requirement of plants.

It was thought advisable to determine the water requirement based on the dry weight of both the aerial portions and the roots of the plants. The water requirement was obtained in this manner for Pride of Saline corn, Blackhull kafir, Dwarf milo, and Dwarf Blackhull kafir. The method used in the isolation of the root systems of these plants has been previously reported by the writer int his Journal.[2]

[1] Briggs, L. J., and Shantz, H. L. The water requirement of plants. I. Investigations in the Great Plains in 1910 and 1911. U. S. Dept. Agr. Bur. Plant Indus. Bul. 284, 49 p., 2 fig., 11 pl. 1913.
——— ——— The water requirement of plants. II. A review of the literature. U. S. Dept. Agr. Bur. Plant Indus. Bul. 285, 96 p., 5 fig. 1913.
——— ——— The relative water requirement of plants. *In* Jour. Agr. Research, v. 3, no. 1, p. 1–64, 1 fig., pl. 1–7. 1914.

[2] Miller, E. C. A comparative study of the root systems and leaf areas of corn and the sorghums. *In* Jour. Agr. Research, v. 6, no. 9, p. 311–332. 1916.

SCREENED INCLOSURE

The plants were grown in a screened shelter in order to protect them from the hailstorms and severe winds that are frequent in this region. The inclosure was 20 feet square and had a flat top 10 feet from the ground. It consisted of a framework of 2 by 4 inch studding spaced 3 feet apart and covered on both the top and sides by a wire netting with a ¼-inch mesh. Cheesecloth was placed around the sides of the inclosure to a height of 4½ feet from the ground. This was held in position by poultry netting tacked over the outside (Pl. LXX, fig. 1).

The bottom of the inclosure was provided with a smooth, rigid floor made of matched pine lumber. The cans were placed in three double rows running north and south inside the inclosure, with a space of 2 feet between each row. The height of the floor was such that the upper surface of the cans came to within 1½ feet of the top of the cheesecloth.

The rate of evaporation inside and outside the shelter was determined by two Livingston [1] porous-cup atmometers. These were renewed every three or four weeks. They were connected with burettes which were graduated to 0.1 c. c., and readings were made twice each day. The atmometer outside the inclosure was placed at a distance of 2 feet from the ground in the center of a plot that was planted to corn. The atmometer in the inclosure was placed a few inches above the upper surface of the cans during the early part of the growing season and 2 feet above their tops when the plants had reached 3 feet in height. The monthly evaporation for the two seasons from the porous-cup atmometers, having a coefficient of 0.74 is given in Table II.

TABLE II.—*Monthly evaporation (in cubic centimeters) inside and outside the screened inclosure for 1914 and 1915*

| Period. | Outside. | Inside. | Ratio. |
|---|---|---|---|
| 1914. | | | |
| June 10 to July 10 | 2,595 | 1,494 | 1.7 |
| July 10 to Aug. 10 | 2,317 | 1,593 | 1.4 |
| Aug. 10 to Sept. 10 | 2,124 | 1,462 | 1.4 |
| 1915. | | | |
| June 10 to July 10 | 2,028 | 1,274 | 1.5 |
| July 10 to Aug. 10 | 1,627 | 1,233 | 1.3 |
| Aug. 10 to Sept. 10 | 1,589 | 976 | 1.6 |

The rate of evaporation within the inclosure as measured by the porous-cup atmometers, was only approximately two-thirds as high as that in the field. Briggs and Shantz [2] found that plants grown in such a shelter had a water requirement approximately 20 per cent lower than

[1] Livingston, B. E. The Relation of Desert Plants to Soil Moisture and to Evaporation. 78 p., illus., Washington, D. C., 1906. (Carnegie Inst. Washington, Pub. 50.) Literature cited, p. 77–78.
——— Operation of the porous-cup atmometer. *In* Plant World, v. 13, no. 5, p. 111–119. 1910.

[2] Briggs, L. J., and Shantz, H. L., 1913. Op. cit.

plants of the same kind grown in the open. The relative water requirment, however, is probably affected little, if at all, by the shading due to an inclosure of this kind, and it offers the only scientific method for studying the relative transpiration of plants under the severe climatic conditions experienced in this region.

### WEIGHING THE CANS

Each can was placed on a small wooden platform, which was provided with a screw eye at either end and mounted on four iron castors. By means of an iron rod, hooked at one end and bent into a handhold at the other, the cans could be moved easily wherever desired (Pl. LXX, fig. 2). The cans were pulled over a track made of pine flooring to a small scale house located 12 feet from the shelter and were weighed every 48 hours on platform scales that were sensitive to 50 gm. (Pl. LXX, fig. 1). In this manner two men could easily weigh the 60 cans in less than 1½ hours.

## EXPERIMENTAL DATA

### CORN

Four varieties of corn were grown in 1914 and three varieties in 1915. The results for the two years are shown in Tables III and IV.

TABLE III.—*Water requirement of Pride of Saline corn at Garden City, Kans., in 1914 and 1915*

| Period of growth. | Pot No. | Number of plants. | Dry matter, including roots. | Dry matter, without root (stem and leaves). | Water transpired. | Water requirement based on— | |
|---|---|---|---|---|---|---|---|
| | | | | | | Total dry weight, including roots. | Total dry weight, excluding roots (stem and leaves). |
| 1914. | | | *Gm.* | *Gm.* | *Kgm.* | | |
| May 26 to Aug. 22. | 13 | 3 | 164. 3 | 150. 6 | 53. 5 | 325. 8 | 355. 4 |
| | 14 | 3 | 169. 8 | 153. 9 | 63. 3 | 373. 1 | 411. 7 |
| | 15 | 3 | 147. 0 | 131. 4 | 61. 7 | 420. 0 | 469. 9 |
| | 16 | 3 | 180. 1 | 163. 7 | 61. 4 | 341. 0 | 375. 3 |
| Mean..... | ..... | ..... | ..... | ..... | ..... | 365±15 | 403±18 |
| 1915. | | | | | | | |
| May 22 to Aug. 25. | 17 | 3 | 236. 1 | 205. 6 | 56. 9 | 241. 1 | 276. 0 |
| | 18 | 3 | 285. 6 | 252. 5 | 64. 2 | 225. 1 | 254. 6 |
| | 19 | 3 | 260. 4 | 234. 4 | 63. 4 | 243. 7 | 270. 7 |
| | 20 | 3 | 230. 6 | 202. 4 | 55. 3 | 240. 0 | 273. 4 |
| | 21 | 3 | 244. 3 | 211. 2 | 58. 1 | 238. 1 | 275. 5 |
| | 22 | 3 | 260. 0 | 228. 3 | 59. 2 | 227. 9 | 259. 6 |
| | 23 | 3 | ..... | 165. 4 | 46. 6 | ..... | 282. 2 |
| | 24 | 3 | ..... | 178. 6 | 46. 0 | ..... | 257. 9 |
| | 25 | 3 | ..... | 180. 5 | 46. 7 | ..... | 259. 0 |
| | 26 | 3 | ..... | 154. 4 | 40. 6 | ..... | 263. 0 |
| Mean..... | ..... | ..... | ..... | ..... | ..... | 236±3 | 267±2 |

TABLE IV.—*Water requirement of Sherrods White Dent, Chinese, and hybrid corn at Garden City, Kans., in 1914 and 1915*

| Variety and period of growth. | Pot No. | Number of plants. | Dry matter, excluding roots (stem and leaves). | Water transpired. | Water requirement. |
|---|---|---|---|---|---|
| 1914. | | | *Gm.* | *Kgm.* | |
| Sherrods White Dent, May 26 to Aug. 22 | 17 | 3 | 133.3 | 54.7 | 410.8 |
| | 18 | 3 | 142.4 | 50.7 | 356.3 |
| | 19 | 3 | 143.7 | 60.8 | 423.3 |
| Mean | | | | | 396±16 |
| Chinese, May 26 to Aug. 22 | 20 | 3 | 136.1 | 58.1 | 427.1 |
| | 21 | 3 | 157.3 | 64.5 | 410.3 |
| Mean | | | | | 418±7 |
| Hybrid $F_3$ $H_{58}$,[a] May 26 to Aug. 22 | 22 | 3 | 120.1 | 40.2 | 335.3 |
| | 23 | 3 | 143.3 | 50.5 | 361.8 |
| | 24 | 3 | 142.6 | 54.4 | 381.4 |
| | 25 | 3 | 155.0 | 51.9 | 342.0 |
| Mean | | | | | 355±8 |
| 1915. | | | | | |
| Sherrods White Dent, May 22 to Aug. 18 | 27 | 3 | 145.7 | 42.8 | 293.7 |
| | 28 | 3 | 150.4 | 43.7 | 291.0 |
| | 29 | 3 | 145.2 | 41.0 | 282.9 |
| | 30 | 3 | 120.5 | 39.8 | 330.9 |
| Mean | | | | | 299±8 |
| Hybrid $F_4$ $H_{58}$,[a] May 22 to Aug. 25 | 43 | 3 | 239.7 | 54.1 | 225.9 |
| | 44 | 3 | 125.6 | 33.3 | 265.7 |
| | 45 | 3 | 137.7 | 36.3 | 264.0 |
| | 46 | 3 | 248.5 | 58.0 | 233.4 |
| | 47 | 3 | 249.3 | 60.7 | 243.7 |
| Mean | | | | | 246±6 |

[a] This hybrid has the following origin: The female parent was a plant belonging to the $F_1$ generation of a cross between Sherrods White Dent corn ♀ and white Chinese corn ♂. The male parent was a plant of the variety known as Esperanza (Mexican corn). The cross was made on the breeding grounds of the Department of Botany of the Kansas Experiment Station in 1910.

Four cans of Pride of Saline corn were grown in 1914 and ten in 1915. These plants varied in mature height from 5 to 6 feet, but produced no ears during either season. The plants grew from May 26 to August 22 in 1914, and from May 22 to August 25 in 1915. The water requirement of Pride of Saline corn, based on the total dry matter, including the roots, was found to be 365±15 in 1914 and 236±3 in 1915. The water requirement, based on the total dry matter of the aerial parts of the plants, was 403±18 and 267±2 for the years 1914 and 1915, respectively (Pl. LXXII, fig. 2).

Sherrods White Dent corn was grown in three cans in 1914 and in four cans in 1915. In 1914 the seeds were planted on May 26 and the

plants were harvested on August 22, while in 1915 they were planted on May 22 and harvested on August 18. The water requirement of this variety of corn, based on the total dry matter of the aerial parts, was found to be 396±16 in 1914 and 299±8 in 1915.

In 1914 two cans were planted to white Chinese corn. The growing season of these plants was from May 26 to August 22. The water requirement, based on the dry weight of the aerial parts, was 418±7.

In 1914 four cans were planted to the $F_3$ generation of a segregate of a hybrid corn developed by the Department of Botany of the Kansas Experiment Station. Five cans of the $F_4$ generation of this hybrid were grown in 1915. Its water requirement, based on the total dry matter of the aerial parts, was 355±8 and 246±6, for the years 1914 and 1915, respectively.

## SORGHUMS

Dwarf milo and Blackhull kafir were the only sorghums grown in 1914. In addition to these two varieties, dwarf black-hulled white kafir, feterita, and sudan grass were grown in 1915. The results for the two seasons are shown in Tables V and VI.

Six cans of Dwarf milo were planted in 1914 and eight cans in 1915. The plants in the former year reached a height of 3 feet, and during the latter year they stood 4½ feet high (Pl. LXXI, fig. 1). The growing season was from May 26 to August 22 in 1914, and from May 22 to September 3 in 1915. The water requirement, based on the total dry matter, including the roots, was found to be 319±5 in the former year and 228±3 in the latter. The water requirement, based on the total dry matter of the aerial parts, was 340±5 and 244±3 for the years 1914 and 1915, respectively. The water requirement, based on the production of grain, was 1,022±100 in 1914 and 508±6 in 1915.

Blackhull kafir was grown in six cans in 1914 and in eight cans in 1915. The seed was planted on May 26 and the plants were harvested on September 3 in 1914, while in 1915 the growing period was from May 22 to September 18. The plants reached a height of 6 feet in each of the growing seasons (Pl. LXXII, fig. 3). The water requirement, based on the total dry matter, including the roots, was 305±6 in 1914 and 204±2 in 1915, while the water requirement, based on the total dry weight of the aerial parts, was 325±7 for the former year and 217±2 for the latter. The water requirement, based on the production of grain, was 1,178±45 in 1914 and 696±19 in 1915.

TABLE V.—*Water requirement of Dwarf milo and Blackhull kafir at Garden City, Kans., in 1914 and 1915*

DWARF MILO

| Period of growth. | Pot No. | Number of plants. | Dry matter, including roots. | Dry matter, without roots. | Grain. | Stem and leaves. | Water transpired. | Water requirement based on— Total dry weight, including roots. | Total dry weight, excluding roots. | Grain. | Stem and leaves. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1914. | | | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Kgm.* | | | | |
| May 26 to August 22.. | 1 | 6 | 199. 0 | 187. 3 | 71. 8 | 115. 5 | 61. 4 | 308. 9 | 328. 2 | 855. 3 | 532. 2 |
| | 2 | 6 | 172. 2 | 161. 5 | 40. 4 | 121. 1 | 55. 8 | 324. 2 | 345. 6 | 1, 381. 9 | 461. 0 |
| | 3 | 6 | 186. 8 | 173. 7 | 45. 0 | 128. 9 | 65. 1 | 348. 7 | 374. 6 | 1, 447. 7 | 505. 4 |
| | 4 | 6 | 196. 4 | 184. 4 | 79. 3 | 105. 1 | 60. 6 | 308. 7 | 328. 7 | 764. 4 | 576. 8 |
| | 5 | 6 | 173. 7 | 161. 7 | 58. 8 | 102. 9 | 51. 6 | 297. 5 | 319. 9 | 879. 0 | 502. 3 |
| | 6 | 6 | 169. 2 | 159. 7 | 68. 5 | 91. 2 | 55. 1 | 326. 0 | 345. 3 | 805. 2 | 604. 8 |
| Mean.. | .... | .... | ..... | ..... | ..... | ..... | ..... | 319±5 | 340±5 | 1022±100 | 530±15 |
| 1915. | | | | | | | | | | | |
| May 22 to September 3... | 1 | 3 | 228. 1 | 214. 6 | 103. 1 | 111. 5 | 51. 5 | 225. 7 | 239. 9 | 499. 5 | 461. 8 |
| | 2 | 3 | 239. 1 | 226. 4 | 114. 6 | 111. 8 | 50. 5 | 211. 5 | 223. 3 | 437. 2 | 452. 3 |
| | 3 | 3 | 245. 4 | 231. 4 | 105. 6 | 125. 8 | 55. 3 | 225. 7 | 239. 3 | 524. 5 | 440. 3 |
| | 4 | 3 | 245. 9 | 223. 3 | 102. 0 | 121. 3 | 56. 1 | 228. 2 | 251. 3 | 549. 1 | 462. 6 |
| | 5 | 3 | 248. 3 | 233. 3 | 109. 6 | 123. 7 | 55. 6 | 224. 1 | 238. 5 | 507. 7 | 449. 8 |
| | 6 | 3 | 231. 6 | 217. 6 | 107. 6 | 110. 0 | 54. 7 | 236. 3 | 251. 5 | 508. 7 | 497. 6 |
| | 7 | 3 | 247. 3 | 230. 5 | 114. 7 | 115. 8 | 60. 4 | 244. 4 | 262. 2 | 526. 7 | 521. 8 |
| | 8 | 3 | 240. 8 | 225. 8 | 108. 3 | 117. 5 | 55. 3 | 229. 8 | 245. 1 | 511. 0 | 471. 0 |
| Mean.. | .... | .... | ..... | ..... | ..... | ..... | ..... | 228±3 | 244±3 | 508±6 | 469±7 |

BLACKHULL KAFIR

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1914. | | | | | | | | | | | |
| May 26 to September 3... | 7 | 4 | 234. 4 | 217. 9 | 54. 5 | 163. 4 | 75. 0 | 320. 0 | 344. 2 | 1, 376. 4 | 459. 1 |
| | 8 | 5 | 247. 0 | 234. 1 | 66. 7 | 167. 4 | 68. 1 | 276. 0 | 291. 2 | 1, 022. 1 | 407. 3 |
| | 9 | 5 | 226. 8 | 212. 6 | 55. 5 | 157. 1 | 67. 7 | 298. 7 | 318. 7 | 1, 220. 9 | 431. 3 |
| | 10 | 6 | 233. 3 | 219. 5 | 60. 5 | 159. 0 | 78. 1 | 335. 0 | 356. 0 | 1, 291. 9 | 491. 5 |
| | 11 | 4 | 186. 5 | 175. 6 | 52. 0 | 123. 6 | 58. 6 | 314. 5 | 334. 1 | 1, 126. 7 | 474. 6 |
| | 12 | 6 | 278. 1 | 257. 3 | 77. 3 | 180. 0 | 79. 8 | 287. 0 | 310. 0 | 1, 032. 4 | 443. 2 |
| Mean.. | .... | .... | ..... | ..... | ..... | ..... | ..... | 305±6 | 325±7 | 1, 178±45 | 451±7 |
| 1915. | | | | | | | | | | | |
| May 22 to September 18.. | 9 | 3 | 360. 7 | 341. 7 | 126. 7 | 215. 0 | 74. 8 | 207. 5 | 219. 1 | 590. 9 | 348. 2 |
| | 10 | 2 | 233. 9 | 219. 3 | 72. 1 | 147. 2 | 49. 2 | 210. 7 | 224. 7 | 683. 6 | 334. 8 |
| | 11 | 3 | 324. 7 | 299. 7 | 92. 4 | 207. 3 | 67. 8 | 208. 9 | 226. 3 | 734. 3 | 327. 3 |
| | 12 | 3 | 311. 3 | 287. 8 | 81. 2 | 206. 6 | 64. 5 | 207. 3 | 224. 2 | 794. 8 | 312. 3 |
| | 13 | 3 | 325. 0 | 310. 3 | 97. 2 | 213. 1 | 67. 9 | 208. 9 | 218. 8 | 698. 5 | 318. 6 |
| | 14 | 3 | 363. 8 | 342. 8 | 89. 6 | 253. 2 | 70. 9 | 194. 9 | 206. 8 | 791. 5 | 280. 0 |
| | 15 | 3 | 353. 9 | 333. 8 | 114. 3 | 219. 5 | 70. 6 | 199. 5 | 211. 5 | 617. 7 | 307. 6 |
| | 16 | 3 | 368. 9 | 354. 2 | 109. 6 | 244. 6 | 72. 0 | 195. 2 | 203. 3 | 657. 3 | 294. 5 |
| Mean.. | .... | .... | ..... | ..... | ..... | ..... | ..... | 204±2 | 217±2 | 696±19 | 315±5 |

TABLE VI.—*Water requirement of Dwarf Blackhull kafir, feterita, and Sudan grass at Garden City, Kans., in 1915*

| Plant and period of growth. | Pot No. | Number of plants. | Dry matter, including roots. | Dry matter, without roots. | Grain. | Stem and leaves. | Water transpired. | Water requirement based on— | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Total dry weight, including roots. | Total dry weight, excluding roots. | Grain. | Stem and leaves. |
| 1915. | | | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Kgm.* | | | | |
| Kafir, Dwarf Blackhull, May 22 to September 11..... | 31 | 3 | 265.7 | 249.7 | 107.0 | 142.7 | 56.4 | 212.3 | 225.9 | 527.3 | 395.4 |
| | 32 | 3 | 235.4 | 221.8 | 88.4 | 133.4 | 48.3 | 205.3 | 217.9 | 546.8 | 362.3 |
| | 33 | 3 | 273.2 | 257.8 | 119.9 | 137.9 | 56.7 | 207.3 | 220.2 | 473.4 | 411.6 |
| | 34 | 2 | 179.2 | 168.8 | 71.7 | 97.1 | 37.5 | 209.7 | 222.6 | 524.1 | 387.0 |
| | 35 | 3 | 247.1 | 230.2 | 95.1 | 135.1 | 50.0 | 202.3 | 217.2 | 525.8 | 370.1 |
| Mean.. | .... | .... | ..... | ..... | ..... | ..... | ..... | 207±2 | 221±2 | 519±8 | 385±6 |
| Feterita, May 22 to September 6... | 36 | 3 | ..... | 175.6 | 59.6 | 116.0 | 42.6 | ..... | 242.7 | 715.2 | 367.5 |
| | 37 | 3 | ..... | 204.7 | 66.0 | 138.7 | 49.4 | ..... | 242.1 | 748.4 | 365.1 |
| | 38 | 3 | ..... | 158.8 | 55.7 | 103.1 | 41.8 | ..... | 263.3 | 750.8 | 405.6 |
| | 39 | 2 | ..... | 143.1 | 42.0 | 101.1 | 35.5 | ..... | 248.2 | 845.7 | 351.3 |
| | 40 | 3 | ..... | 182.6 | 52.4 | 130.2 | 45.3 | ..... | 248.3 | 865.2 | 348.2 |
| Mean.. | .... | .... | ..... | ..... | ..... | ..... | ..... | ..... | 249±2 | 785±24 | 367±6 |
| Sudan grass, May 22 to September 14..... | 41 | 5 | ..... | 186.4 | 33.2 | 153.2 | 52.5 | ..... | 281.6 | 1,581.3 | 342.6 |
| | 42 | 5 | ..... | 173.6 | 28.4 | 145.2 | 50.7 | ..... | 292.5 | 1,788.3 | 349.7 |
| | 43 | 5 | ..... | 176.4 | 42.5 | 133.9 | 60.5 | ..... | 343.3 | 1,425.2 | 452.3 |
| Mean.. | .... | .... | ..... | ..... | ..... | ..... | ..... | ..... | 306±15 | 1,598±76 | 381±28 |

Dwarf Blackhull kafir was grown only in 1915. The growing season for these plants was from May 22 to September 11. The water requirement, based on the total dry matter, including the roots, was 207±2, and based on the total dry weight of the aerial portions, was 221±2. The water requirement, based on the production of grain, was 519±8 (Pl. LXXI, fig. 2).

Feterita was grown in five cans in 1915. The seed was planted on May 22 and the plants were harvested on September 6. The water requirement, based on the total dry matter of the aerial parts, was 249±2, while the water requirement, based on the seed production was 785±24 (Pl. LXXI, fig. 3).

Three cans were planted to Sudan grass in 1915. These plants reached a height of 6 feet during the growing period from May 22 to September 14 (Pl. LXXII, fig. 1). The water requirement, based on the dry weight of the aerial parts, was 306±15 and, based on the production of grain, was 1598±76.

## SUMMARY

The water requirement was determined for four varieties of corn and two varieties of sorghum in 1914 and for three varieties of corn and five varieties of sorghum in 1915.

The plants were grown in large sealed galvanized-iron cans which contained approximately 110 kgm. of soil. The soil had a wilting coefficient of 13, and under the conditions of the experiment it had a moisture content of 20 to 21 per cent (dry basis). This moisture content was kept approximately constant by replacing every 48 hours the water that had been lost by transpiration.

Three plants of corn were grown in each can during both seasons. Six sorghum plants were grown to each can in 1914, but in 1915 the number of plants was reduced to three plants to a can.

The plants were grown in a screened inclosure in order to protect them from the hailstorms and severe winds that are prevalent in western Kansas. The rate of evaporation in such a shelter was found to be only two-thirds as high as under field conditions.

The season of 1915 was cooler and more humid, and the rate of evaporation much lower than in 1914. As a consequence the water requirement of the former year was only about 66 per cent of that of the latter year. A summary of the water requirement for the two seasons is given in Table VII.

TABLE VII.—*Summary of the water requirement of the varieties of corn and sorghum grown at Garden City, Kans., in 1914 and 1915*

| Plant and period of growth. | Water requirement based on— | | | |
|---|---|---|---|---|
| | Dry matter, including roots. | Dry matter, excluding roots. | Grain. | Stem and leaves. |
| 1914. | | | | |
| CORN: | | | | |
| Pride of Saline, May 26 to August 22 | 365±15 | 403±18 | ..... | 403±18 |
| Sherrods White Dent, May 26 to August 22 | ..... | 396±16 | ..... | 396±16 |
| Hybrid $F_3$ $H_{58}$, May 26 to August 22 | ..... | 355± 8 | ..... | 355± 8 |
| Chinese, May 26 to August 22 | ..... | 418± 7 | ..... | 418± 7 |
| KAFIR: | | | | |
| Blackhull, May 26 to September 3 | 305± 6 | 325± 7 | 1,178± 45 | 451± 7 |
| MILO: | | | | |
| Dwarf, May 26 to August 22 | 319± 5 | 340± 5 | 1,022±100 | 530±15 |
| 1915. | | | | |
| CORN: | | | | |
| Pride of Saline, May 22 to August 25 | 236± 3 | 267± 2 | ..... | 267± 2 |
| Sherrods White Dent, May 22 to August 18 | ..... | 299± 8 | ..... | 299± 8 |
| Hybrid $F_4$ $H_{58}$, May 22 to August 25 | ..... | 246± 6 | ..... | 246± 6 |

TABLE VII.—*Summary of the water requirement of the varieties of corn and sorghum grown at Garden City, Kans., in 1914 and 1915*—Continued

| Plant and period of growth. | Water requirement based on— | | | |
|---|---|---|---|---|
| | Dry matter, including roots. | Dry matter, excluding roots. | Grain. | Stem and leaves. |
| 1915. | | | | |
| KAFIR: | | | | |
| Blackhull, May 22 to September 18. | 204± 2 | 217± 2 | 696± 19 | 315± 5 |
| Dwarf Blackhull, May 22 to September 11 | 207± 2 | 221± 2 | 519± 8 | 385± 6 |
| MILO: | | | | |
| Dwarf, May 22 to September 3 | 228± 3 | 244± 3 | 508± 6 | 469± 7 |
| FETERITA: | | | | |
| May 22 to September 6 | ..... | 249± 2 | 785± 24 | 367± 6 |
| SUDAN GRASS: May 22 to September 14. | ..... | 306±15 | 1,598± 76 | 381±28 |

Using the water requirement of Blackhull kafir as 1, the water requirement of the plants grown in 1914 would be as follows: Dwarf milo 1.04, hybrid corn 1.09, Sherrods White Dent corn 1.22, and Pride of Saline corn 1.24. In 1915, if the water requirement of Blackhull kafir be considered as 1, the water requirement of Dwarf Blackhull kafir would be 1.02; Dwarf milo, 1.12; feterita, 1.14; hybrid corn, 1.17; Pride of Saline corn, 1.23; Sherrods White Dent corn, 1.37; and Sudan grass, 1.41.

## PLATE LXX

Fig. 1.—General view of the screened inclosure and the scale house.

Fig. 2.—Method of moving the cans.

Fig. 3.—General view of the plant shelter and the surrounding country at Garden City, Kans.

## PLATE LXXI

Fig. 1.—Dwarf milo, grown May 22 to September 3, 1915. Water requirement based on total dry matter, including roots, 228±3. Based on dry matter, excluding roots, 244±3. Average of 8 cans.

Fig. 2.—Dwarf Blackhull kafir, grown May 22 to September 11, 1915. Water requirement based on total dry matter, including roots, 207±2. Based on total dry matter, excluding roots, 221±2. Average of 5 cans.

Fig. 3.—Feterita, grown May 22 to September 6, 1915. Water requirement based on total dry matter, excluding roots, 249±2. Average of 5 cans.

## PLATE LXXII

Fig. 1.—Sudan grass, grown May 22 to September 14, 1915. Water requirement based on total dry matter, excluding roots, 306±15. Average of 3 cans.

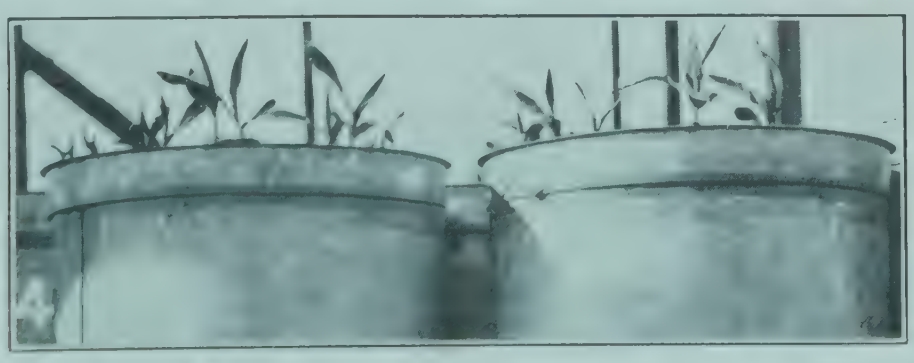
Fig. 2.—Pride of Saline corn, grown May 22 to August 25, 1915. Water requirement based on total dry matter, including roots, 236±3. Based on total dry matter, excluding roots, 267±2. Average of 10 cans.

Fig. 3.—Blackhull kafir, grown May 22 to September 18, 1915. Water requirement based on total dry matter, including roots, 204±2. Based on total dry matter, excluding roots, 217±2. Average of 8 cans.

Fig. 4.—Method of sealing the lids with tape and the wax seal around the plants.

2

3


4

# AVAILABILITY OF MINERAL PHOSPHATES FOR PLANT NUTRITION [1]

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## INTRODUCTION

Phosphorus is the key to permanent systems of agriculture for a large portion of the common soils of the corn belt. These soils contain, as an average, 5,000 pounds of nitrogen, 1,200 pounds of phosphorus, and 35,000 pounds of potassium for the surface soil to the depth of 6⅔ inches. If the land were producing corn at the rate of 100 bushels per acre, the nitrogen would be sufficient for 50 crops, the phosphorus for 70 crops, and the potassium for about 1,842 crops. The nitrogen supply can be maintained by the growth and judicious management of leguminous crops. Potassium is present in quantities adequate for many years. With phosphorus the problem is different. This element can not be gathered from the soil air by legumes; nor is it one of unlimited supply. When once removed, phosphorus must be returned to the land in crop residues, in farm manures, or in commercial fertilizers which contain phosphorus.

Since the introduction of commercial fertilizers, more or less discussion has been carried on concerning the value of insoluble mineral phosphates as a source of phosphorus for the nutrition of plants. In Europe (28, p. 329)[3] the highest authorities on agricultural problems have discouraged the use of insoluble phosphates, while in America scientists and practical men have disagreed. Investigations which have been conducted on the use of insoluble minerals are by no means conclusive. Therefore it is the purpose of the work reported in the following pages to throw more light on this question, which is of so great economic importance and scientific significance. The subject matter will be presented according to the following divisions:

I. Review of literature regarding the availability of phosphate minerals.

II. The availability of phosphorus in Tennessee brown rock phosphate for wheat (*Triticum vulgare*), oats (*Avena sativa*), rye (*Secale cereale*), barley (*Hordeum sativum hexastichon*), cowpeas (*Vigna catjang*), soybeans (*Glycine hispida*), timothy (*Phleum pratense*), red clover (*Trifolium pratense*), and alfalfa (*Medicago sativa*).

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[2] The author wishes to express his appreciation for the suggestions and encouragement tendered by Dr. C. G. Hopkins and Dr. A. L. Whiting, of the Illinois Experiment Station.

[3] Reference is made by number to "Literature cited," p. 513–514.

III. A comparative study of the productive powers of six mineral phosphates for farm crops.

IV. The influence of fermenting dextrose and crop residues on the availability of phosphorus in finely ground rock phosphate.

V. The influence of the size of particles on the availability of phosphorus in mineral phosphates.

## REVIEW OF LITERATURE

The availability of mineral phosphates for plant nutrition has been under investigation at various institutions for more than half a century. Among the earlier scientists who attempted to determine the availability of the phosphorus in mineral phosphates was Dyer (4), who found that undissolved phosphate produced better returns than dissolved phosphate for swedes and oats. Frear (5) studied the comparative value of various phosphorus carriers for farm crops. Finely ground bone and reverted phosphate produced the largest number of mature stalks of corn, and finely ground bone, the highest yield of ears. Superphosphate and certain mineral or raw phosphates were put in field trials by Johnson (9), and for corn, dissolved bone black was superior to others tested. Bishop (1) grew soybeans in pot cultures and concluded that concentrated phosphate and acid phosphate were more desirable than Florida soft rock and iron and aluminum phosphate. Equivalent amounts of different carriers of phosphate were employed by Hess (7) in a 4-year rotation of corn, oats, wheat, and grass. Finely ground bone gave the highest yields of wheat, with raw rock second. Ground bone was most effective for corn, while for oats insoluble ground bone seemed to be satisfactory. South Carolina rock was very useful for clover. Jordan (10) conducted two experiments at the Maine Station with different forms of phosphate. In the first experiment the minerals were applied in equal quantities. For the first two years the acid phosphate gave the highest returns, but later bone meal took the lead. Raw rock was only about half as productive as the other two. In the second trial equal money values of phosphates were applied; and the author points out that, with but one exception, the raw rock gave larger returns than acid phosphate. The work of Jordan, previously mentioned, was continued by Merrill (15), who used pure sand cultures in the greenhouse. Two facts are clear from Merrill's work. First, plants differ widely in their power to assimilate phosphorus from different phosphates. Second, turnips and rutabagas gave almost as good results with raw rock phosphate as with acid phosphate. Later, at the New York Station, Jordan (11) continued the work which he had begun at the Maine Station. His results are in accord with the work previously reported by himself and Merrill.

In 1890 Goessman (2) outlined what has since become a most extensive investigation, concerning the availability of phosphate minerals. In reporting on this work Brooks says (3, p. 104) that—

> It is possible to produce profitable crops of most kinds by liberal use of natural phosphates, and in a long series of years there might be a considerable money saving in depending, at least in part, upon these rather than upon the higher-priced dissolved phosphates.

Results from a second series of experiments begun in Massachusetts in 1897, along the same line as that outlined by Goessman, indicate that phosphatic slag was "exceedingly available for crops, but the Florida soft phosphate was very inferior. For certain crops, South Carolina rock gave surprisingly good returns * * *."

Prianishnikov (20) states that lupins and peas have a very marked ability to obtain phosphorus from natural phosphate, while wheat and oats must be assisted by the solvent powers of the soil or they can not produce normal crops. Schloessing (22) concludes from his experiments that it is not necessary that phosphate should be in a state of solution, since the roots of plants are able to dissolve the phosphorus compounds without the intervention of water.

Patterson (18) reports results, based on a study of various phosphates, which indicate that reverted phosphate gave the highest average yield for corn, wheat, and hay. South Carolina rock phosphate produced slightly better yields than bone black, and Florida soft rock phosphate was quite available for wheat. Wheeler and Adams (30, 31, 32) found raw phosphate profitable for peas, oats, crimson clover, and Japanese millet when used on unlimed land; but for flat turnips, beets, and cabbage it gave poor yields. They are of the opinion that rock phosphate is likely to be most useful when applied to moist soils rich in organic matter, where legumes, corn, and "possibly wheat and oats are to be grown."

Thorne (24, 25), of Ohio, in 1897 inaugurated a very extensive study of the comparative value of raw rock phosphate and acid phosphate used in conjunction with manure. Where, in computing the yields of corn, wheat, and clover, he took the average of all the unfertilized plots as a basis for comparison, he reports (24, p. 18)—

> By this method of calculation the average increase on Plots 2 [floats plus yard manure] and 3 [floats plus stall manure] combined is found to be practically the same as that on Plots 5 [acid phosphate plus yard manure] and 6 [acid phosphate plus stall manure] combined, but when the larger cost of the acid phosphate is deducted the net gain is a little greater on Plots 2 and 3 [with raw phosphate].

By another method of computing the increase he obtains results less favorable to raw phosphate.

Truog (27) has demonstrated rather clearly that farm crops are variable in their ability to secure phosphorus from different sources. Nine of the ten crops tested by him made a better growth on aluminum phos-

phate than on calcium phosphate, and "six made better growth on iron phosphate than on calcium phosphate."

Under the direction of Hopkins (8), the Illinois Experiment Station is conducting probably the most extensive investigation of any in the world on the use of rock phosphate. Some of the most interesting results were obtained from a field near Galesburg, Knox County, Ill., on brown silt loam prairie soil.

> Phosphorus applied in fine-ground natural rock phosphate in part as top dressing, and with no adequate provision for decaying organic matter, paid only 47 per cent on the investment as an average of the first three years. But it should be kept in mind that the word *investment* is here used in its proper sense, for the phosphorus that was removed in the increase produced was less than 2 per cent of the amount applied, and that removed in the total crops less than one-third. During the last six years, however, the phosphorus has paid 130 per cent on the investment, even though two-thirds of the application remains to positively enrich the soil (8, p. 15).

Newman (16) investigated the use of floats with and without cottonseed meal. He found a marked increase in availability where organic matter was used in conjunction with the mineral phosphate. Later experiments by Newman and Clayton (17) confirmed the above results. Lupton (13) continued the work of Newman, but used acid phosphate as a check on the raw rock phosphate, both with and without organic matter. His results are also in accord with Newman's earlier experiments. Where floats were mixed with cottonseed meal and allowed to ferment, the data seemed to show that the fermentation of the material had very little, if any, influence on the availability of the phosphate. Pfeiffer and Thurman (19) found no beneficial results from composting raw rock phosphate with decaying organic matter. In Canada (23) fermenting manures were found to have only slightly solvent action on composted rock phosphate.

Hartwell and Pember (6) mixed fresh cow manure and floats and allowed them to ferment. They feel that there was practically no increase in the availability of phosphorus in the floats. McDowell (14) also found no increase in the availability of phosphate in finely ground rock phosphate by composting the mineral with cow and horse manure. Tottingham and Hoffmann (26), following the same line of investigation as that which McDowell observed, actually found a decrease in water-soluble phosphorus, but the results were similar with acid phosphate.

Krober (12) was unable to find any increase in availability of mineral phosphates by composting with sawdust and allowing fermentation to proceed. Truog (27) believes that fermented manure has a slightly solvent action on crude phosphate. He also points out that a uniform distribution of the phosphate in the soil will give much better results than that poorly distributed.

Krober (12) shows that the acid-forming bacteria and yeasts are of great value in rendering some of the phosphorus in insoluble phosphate

available. He makes the statement that carbon dioxid was more active than other acids in this respect.

The degree of fineness plays an important part in the availability of the crude phosphates. Jordan (11) proves this quite conclusively. He procured better results from the phosphates which were ground to an impalpable powder. Analysis of the plants showed an increase in the proportion of dry matter to phosphorus as the size of the particles decreased. Voelcker (29) in some of the earliest work says that the efficiency of insoluble calcium phosphate depends upon the minuteness of division; the finer the particles the more energetic will be its action.

## EXPERIMENTAL WORK

### MEDIUM FOR PLANT GROWTH

Pure white sand was used throughout these experiments as a medium for plant growth. For most of the work this material was leached with a dilute solution of hydrochloric acid for three days to insure the removal of plant food. The sand was then washed with distilled water until there was no trace of acid in the drainage solution. Next it was placed on clean paper until dry, when it was sifted, in order that foreign particles might be removed. Samples were collected for a phosphorus determination from each lot of sand washed, but in no case during the progress of the study was the slightest trace of phosphorus detected.

### POTS

Two sizes of pots were used in this investigation. When it was necessary to grow the crop to maturity, the small glass battery jars, approximately 6 inches in diameter and 8 inches in height, proved very satisfactory; but when a grain crop was desired, the 4-gallon stone pots were more suitable. All jars were supplied with adequate drainage.

For the cultures grown in the winter the pots were covered with a coat of black paint, but for the summer series a white coat was placed over the black. The black paint prevented the growth of algæ and the white had a tendency to keep the temperature from becoming excessive within the jars. This precaution was clearly justified, for upon several occasions there was a difference of 5° to 10° in temperature between the black and white pots.

### KINDS OF CROPS GROWN

Wheat, oats, rye, barley, timothy, cowpeas, soybeans, clover, and alfalfa—nine common crops that are cultivated on Illinois farms—were grown under various treatments for this investigation. High-grade seed from the previous season's crop was selected for planting, and in all cases the grains were treated with a solution of formalin to prevent smut.

In planting the seed special care was exercised in order to obtain a perfect stand, and in only a few instances was there a failure to get the proper number of plants for each pot. It seems in keeping with accurate methods of research to plant more seeds per pot than would be required for a perfect stand if they all germinated. It is safer to remove the extra plants than to transplant or reseed, and the plants are more likely to be uniform if it is possible to make some choice in thinning them down. An exact record was kept of the number of seeds planted, and all those which failed to germinate were dug out.

For inoculating the legumes, nodules from the same crop as the plant to be infected were crushed and placed in 1 liter of distilled water, and 10 c. c. of this solution were applied to the zone nearest the seed. If the nodules were not available, 300 gm. of soil from a field where the respective legumes had been grown were well shaken with 500 c. c. of water, filtered, and 10 c. c. of this solution were applied in the same manner as indicated above.

## PLANT FOODS

The first application of plant food was made when the crops were planted, the others at intervals of two weeks. The plant foods were made up in the following manner:

Nitrogen: Dissolved 80 gm. of ammonium nitrate, 50 gm. of potassium sulphate, and 20 gm. of magnesium sulphate each in 2,500 c. c. of distilled water, and 0.1 gm. of ferric chlorid in 250 c. c. of distilled water. A standard application of these plant foods was 10 c. c. of each of the first three and 1 c. c. of the last diluted as desired. In no case was the solution applied in a concentrated form.

## MOISTURE SUPPLY

Throughout the first period of these experiments, the water content of the sand was maintained at 14 per cent by weighing the jar each week. This phase of the details became so burdensome that it was omitted. The method was not accurate, at least during the latter period of growth, because of the irregularity in plant development due to different treatments. Some pots gave off more than 10 times the quantities transpired from others. Satisfactory results were obtained by watering the pots when they needed a supply of moisture and no difficulty was experienced in determining the point where the water content of the sand was below normal.

Whenever weather conditions would permit, the pot cultures were placed on trucks and removed to the cage out of doors.

## TIME OF HARVESTING AND HANDLING THE CROP

The time of harvest was governed largely by the condition of the experiment. However, in most instances the same factors which control the time of harvest in general farm practice held true here. The grain

crops developed to full maturity, while the clover and alfalfa were cut for hay. Cowpeas and soybeans grown during the winter months were cut for hay, but those planted in the spring produced a seed crop.

Complete data on time of blooming, time of heading, number of plants, number of stems, and height of plants were collected for a comparison which might be of value in interpreting results, although such records will be omitted from this paper. The total weight of grain and straw, together with photographs, will suffice for drawing conclusions.

After harvesting the pot cultures, the materials were suspended in cheesecloth bags from the roof of the greenhouse for a period of two weeks. This was sufficient time for the product to come to a constant air-dried condition. Usually two weighings at an interval of two days were made as a check to insure accurate results.

## ANALYSIS

The plants were first cut fine and then ground in a steel mill until the particles would pass a sieve of 80 meshes to the inch. Next, the materials were thoroughly mixed and samples taken for analytical purposes.

The method for the determination of phosphorus was essentially the Pemberton outline, with slight modifications.

Two gm. of the sample [1] were weighed out and moistened with calcium acetate. The sample was then dried in an electric oven and afterwards transferred to a muffle and there remained until the product was burned to a white ash. The ash was taken up with 5 c. c. of nitric acid and heated on a water bath for several minutes. It was necessary to filter to remove any silica present. From this point the regular procedure followed in the volumetric method was observed.

The mineral phosphates used in this investigation represent six types from different sections of the United States and Canada. The total phosphorus and the phosphorus soluble in citric acid are reported in Table I.

TABLE I.—*Total phosphorus and citric-acid-soluble phosphorus in various kinds of rock phosphate* [2]

| Kinds of phosphate. | Phosphorus. | |
|---|---|---|
| | Total. | Soluble in citric acid. |
| Tennessee brown rock phosphate | 12.75 | 9.92 |
| Tennessee blue rock phosphate | 13.40 | 10.29 |
| Utah rock phosphate | 13.81 | 8.66 |
| South Carolina land rock phosphate | 13.75 | 6.89 |
| Florida soft rock phosphate | 13.98 | 10.55 |
| Canadian apatite | 12.75 | 5.57 |

[1] Two gm. was satisfactory for straw and hay, but for the grain ¼ gm. was sufficient.

[2] Four gm. of each of the mineral phosphates were placed in a 1-liter flask and then 1,000 c. c. of a 0.2 per cent solution of citric acid was poured on the ground rock, where it remained for 48 hours with occasional shaking. Then some of the solution was filtered and 100 c. c. of the filtrate taken for analysis.

## AVAILABILITY OF THE PHOSPHORUS IN TENNESSEE BROWN ROCK PHOSPHATE

This series comprises a study of the ability of different crops to secure phosphorus for growth from Tennessee brown rock phosphate without the aid of decaying organic matter. The literature indicates rather clearly that crops differ widely in this respect, but there is but very little direct information from trials conducted under controlled conditions where sand was used as a substitute for soil. The suggestion has been made, also, that there is slight increase in the yield with large application of phosphate. The object of the series reported in Tables II to VI is to present new information on these two important points.

The pots used were the large, glazed 4-gallon jars into which could be placed 22,000 gm. of sand (Pl. LXXIII, LXXIV, LXXV). In this case the sand was not leached with dilute acid, but was washed for several days with distilled water. The rock phosphate was ground sufficiently fine to pass through a sieve of 100 meshes to the inch. On March 20, 1914, the pots were seeded; and after the plants had made satisfactory growth, they were thinned to 15 to each jar.

TABLE II.—*Dry matter and phosphorus content of plant products from wheat and oats*

SERIES 1A; SPRING WHEAT HARVESTED ON JUNE 29, 1914[a]

| Pot No. | Phosphate added. | Grain. | Straw. | Phosphorus. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Grain. | Straw. | Grain. | Straw. | Total in grain and straw. | Percentage removed. |
| | *Gm.* | *Gm.* | *Gm.* | *Per cent.* | *Per cent.* | *Mgm.* | *Mgm.* | *Mgm.* | |
| 1.... | 0 | 0 | 6.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 2.... | 0 | 0 | 6.5 | ..... | ..... | ..... | ..... | ..... | ..... |
| 3.... | 11 | 1.0 | 16.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 4.... | 11 | 1.4 | 19.1 | 0.260 | 0.038 | 3.64 | 7.26 | 10.90 | 0.78 |
| 5.... | 22 | 4.1 | 21.9 | ..... | ..... | ..... | ..... | ..... | ..... |
| 6.... | 22 | 4.0 | 20.6 | .257 | .029 | 10.31 | 6.06 | 16.37 | .58 |
| 7.... | 66 | 12.7 | 39.3 | ..... | ..... | ..... | ..... | ..... | ..... |
| 8.... | 66 | 12.8 | 35.9 | .240 | .019 | 30.72 | 6.64 | 37.36 | .44 |
| 9.... | 220 | 17.5 | 42.9 | ..... | ..... | ..... | ..... | ..... | ..... |
| 10... | 220 | 16.8 | 40.4 | .335 | .026 | 56.20 | 10.30 | 66.50 | .24 |

SERIES 1B; SIXTY-DAY OATS HARVESTED ON JUNE 7, 1914[b]

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 11... | 0 | 0 | 6.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 12... | 0 | 0 | 6.1 | ..... | .035 | ..... | 2.14 | 2.14 | ..... |
| 13... | 11 | 4.7 | 10.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 14... | 11 | 4.5 | 10.7 | 0.184 | .038 | 8.28 | 4.11 | 12.38 | .88 |
| 15... | 22 | 7.6 | 13.6 | .189 | .032 | 14.36 | 4.35 | 18.72 | .67 |
| 16... | 22 | 7.2 | 14.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 17... | 66 | 10.9 | 18.6 | ..... | ..... | ..... | ..... | ..... | ..... |
| 18... | 66 | 12.1 | 15.4 | .229 | .038 | 27.71 | 5.85 | 33.56 | .40 |
| 19... | 220 | 16.8 | 22.9 | ..... | ..... | ..... | ..... | ..... | ..... |
| 20... | 220 | 14.3 | 20.0 | .354 | .059 | 50.62 | 11.80 | 62.42 | .22 |

[a] Seed planted in each pot in series 1A contained 0.46 per cent of phosphorus. Fifteen seeds contained 1.7 mgm. of phosphorus.

[b] Seed planted in each pot in series 1B contained 0.35 per cent of phosphorus. Fifteen seeds contained 1.29 mgm. of phosphorus.

TABLE III.—*Dry matter and phosphorus content of plant products from timothy and red-clover hay*

SERIES 1E; TIMOTHY HARVESTED ON JULY 21, SEPT. 26, AND NOV. 25, 1914

| Pot No. | Phosphate added. | Crop. | | | Phosphorus. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | First cutting. | Second cutting. | Third cutting. | First cutting. | Second cutting. | First cutting. | Second cutting. | Total, two crops. | Percentage removed. |
| | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Per cent.* | *Mgm.* | *Per cent.* | *Mgm.* | *Mgm.* | |
| 41 | 0 | 0.5 | 1.0 | 1.8 | ..... | ..... | ..... | ..... | ..... | ..... |
| 42 | 0 | .8 | .6 | 1.5 | ..... | ..... | ..... | ..... | ..... | ..... |
| 43 | 11 | 11.8 | 8.6 | 4.3 | ..... | ..... | ..... | ..... | ..... | ..... |
| 44 | 11 | 12.8 | 7.1 | 4.5 | ..... | ..... | ..... | ..... | ..... | ..... |
| 45 | 22 | 17.2 | 10.0 | 6.0 | 0.067 | 0.102 | 11.52 | 10.15 | 21.67 | 0.77 |
| 46 | 22 | 17.1 | 9.6 | 6.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 47 | 66 | 27.5 | 26.0 | 7.9 | ..... | ..... | ..... | ..... | ..... | ..... |
| 48 | 66 | 28.0 | 25.6 | 8.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 49 | 220 | 31.6 | 28.0 | 8.2 | [a].126 | .170 | 37.80 | 49.98 | 87.78 | .31 |
| 50 | 220 | 27.7 | 30.8 | 8.7 | ..... | ..... | ..... | ..... | ..... | ..... |

SERIES 1H; RED-CLOVER HAY HARVESTED ON JULY 20, SEPT. 26, DEC. 25, 1914

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 71 | 0 | .4 | .2 | .1 | ..... | ..... | ..... | ..... | ..... | ..... |
| 72 | 0 | .3 | .1 | .1 | ..... | ..... | ..... | ..... | ..... | ..... |
| 73 | 11 | 5.1 | 3.5 | 3.4 | ..... | ..... | ..... | ..... | ..... | ..... |
| 74 | 11 | 4.0 | 2.8 | 3.5 | ..... | ..... | ..... | ..... | ..... | ..... |
| 75 | 22 | 7.8 | 12.2 | 8.4 | .041 | .055 | 3.23 | 6.73 | 9.967 | .36 |
| 76 | 22 | 9.0 | 12.8 | 8.7 | ..... | ..... | ..... | ..... | ..... | ..... |
| 77 | 66 | 16.2 | 23.0 | 13.3 | ..... | ..... | ..... | ..... | ..... | ..... |
| 78 | 66 | 20.8 | 24.1 | 13.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 79 | 220 | 42.8 | 36.5 | [b]10.0 | .169 | [c].215 | 72.55 | 78.29 | 150.839 | .54 |
| 80 | 220 | 54.5 | 37.8 | 14.2 | ..... | ..... | ..... | ..... | ..... | ..... |

[a] The phosphorus content of average timothy hay is 0.09 per cent.
[b] Attacked by worms.
[c] The phosphorus content of average red-clover hay is about 0.21 per cent.

TABLE IV.—*Dry matter and phosphorus content of plant products from cowpeas and soybeans*

SERIES 1F; COWPEAS HARVESTED ON JULY 6, 1914

| Pot No. | Phosphate added. | Grain. | Straw. | Phosphorus. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Grain. | Straw. | Grain. | Straw. | Total in grain and straw. | Percentage removed. |
| | *Gm.* | *Gm.* | *Gm.* | *Per cent.* | *Per cent.* | *Mgm.* | *Mgm.* | *Mgm.* | |
| 51 | 0 | 0 | 2.8 | ..... | ..... | ..... | ..... | ..... | ..... |
| 52 | 0 | 0.3 | 2.7 | ..... | 0.073 | ..... | 1.97 | 1.97 | ..... |
| 53 | 11 | 0.7 | 4.3 | ..... | ..... | ..... | ..... | ..... | ..... |
| 54 | 11 | 0 | 4.9 | ..... | .095 | ..... | 4.66 | 4.66 | ..... |
| 55 | 22 | 1.4 | 7.6 | 0.273 | .097 | 3.82 | 7.37 | 11.19 | 0.40 |
| 56 | 22 | 0.7 | 6.7 | ..... | ..... | ..... | ..... | ..... | ..... |
| 57 | 66 | 11.7 | 23.8 | .300 | .117 | 35.08 | 27.92 | 62.99 | .75 |
| 58 | 66 | 12.1 | 27.6 | ..... | ..... | ..... | ..... | ..... | ..... |
| 59 | 220 | 12.1 | 22.5 | ..... | ..... | ..... | ..... | ..... | ..... |
| 60 | 220 | 14.1 | 30.9 | [a].297 | .128 | 41.86 | 39.40 | 81.26 | .29 |

[a] Seed planted in each pot contained 0.434 per cent of phosphorus. Fifteen cowpea seeds contained 11.7 mgm. of phosphorus.

TABLE IV.—*Dry matter and phosphorus content of plant products from cowpeas and soybeans*—Continued.

SERIES 1G; SOYBEANS HARVESTED ON JUNE 10, 1914

| Pot No. | Phosphate added. | Grain. | Straw. | Phosphorus. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Grain. | Straw. | Grain. | Straw. | Total in grain and straw. | Percentage removed. |
| | *Gm.* | *Gm.* | *Gm.* | *Per cent.* | *Per cent.* | *Mgm.* | *Mgm.* | *Mgm.* | |
| 61 | 0 | 1.0 | 9.0 | ..... | ..... | ..... | ..... | ..... | ..... |
| 62 | 0 | 1.0 | 8.3 | 0.360 | 0.058 | 3.60 | 4.77 | 8.37 | ..... |
| 63 | 11 | 2.0 | 9.2 | ..... | ..... | ..... | ..... | ..... | ..... |
| 64 | 11 | 2.8 | 10.2 | .359 | .045 | 10.06 | 4.54 | 14.60 | 1.04 |
| 65 | 22 | 3.5 | 13.5 | ..... | ..... | ..... | ..... | ..... | ..... |
| 66 | 22 | 2.4 | 10.8 | .448 | .062 | 10.76 | 6.64 | 17.40 | .62 |
| 67 | 66 | 2.9 | 14.9 | ..... | ..... | ..... | ..... | ..... | ..... |
| 68 | 66 | 3.4 | 15.3 | .449 | .061 | 15.25 | 9.38 | 24.63 | .29 |
| 69 | 220 | 4.7 | 15.2 | ..... | ..... | ..... | ..... | ..... | ..... |
| 70 | 220 | 4.2 | 13.4 | [a].448 | .088 | 18.83 | 11.83 | 30.66 | .11 |

[a] Seed planted in each pot contained 0.6 per cent of phosphorus. Fifteen soybean seeds contained 11.9 mgm. of phosphorus.

TABLE V.—*Dry matter and phosphorus content of plant products from alfalfa harvested on June 4, July 18, Sept. 26, and Nov. 11, 1914—series 11*

| Pot No. | Phosphate added. | First cutting. | Second cutting. | Third cutting. | Fourth cutting. | Phosphorus.[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | First cutting. | Second cutting. | Third cutting. | First cutting. | Second cutting. | Third cutting. | Total, three cuttings. | Percentage removed from pots. |
| | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Per ct.* | *Per ct.* | *Per ct.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Per ct.* |
| 81 | 0 | 0.3 | 0.2 | 0.5 | 0.4 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 82 | 0 | .3 | .2 | .6 | .4 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 83 | 11 | 5.5 | 8.9 | 13.0 | 6.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 84 | 11 | 7.1 | 9.0 | 12.7 | 6.5 | 0.17 | 0.19 | 0.18 | 22 | 25 | 22 | 69.9 | 2.49 |
| 85 | 22 | 13.0 | 13.0 | 12.5 | 5.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 86 | 22 | 11.0 | 11.0 | [b] 10.0 | 5.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 87 | 66 | 13.6 | 13.9 | 18.6 | 10.2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 88 | 66 | 13.6 | 12.8 | 16.5 | 10.1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 89 | 220 | 18.6 | 19.9 | 20.0 | 10.7 | .10 | .26 | .28 | .17 | 51 | 55 | 124.6 | .44 |
| 90 | 220 | 17.1 | 15.6 | 16.0 | 10.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

[a] Alfalfa hay contains 0.0172 per cent of phosphorus. [b] Attacked by worms.

TABLE VI.—*Dry matter produced by spring rye and barley—series 1C*

| Pot No. | Rye. | | | Pot No. | Barley. | | |
|---|---|---|---|---|---|---|---|
| | Phosphate added. | Grain. | Straw. | | Phosphate added. | Grain. | Straw. |
| | *Gm.* | *Gm.* | *Gm.* | | *Gm.* | *Gm.* | *Gm.* |
| 21 | 0 | 0 | 1.9 | 31 | 0 | 0 | 8.7 |
| 22 | 0 | 0 | 1.8 | 32 | 0 | 0 | 7.2 |
| 23 | 11 | 0 | 10.9 | 33 | 11 | 2.6 | 20.3 |
| 24 | 11 | 0 | 11.6 | 34 | 11 | 1.3 | 16.4 |
| 25 | 22 | 0 | 22.2 | 35 | 22 | 2.0 | 17.8 |
| 26 | 22 | 0 | 21.0 | 36 | 22 | 8.5 | 22.3 |
| 27 | 66 | 0 | 37.0 | 37 | 66 | 5.0 | 23.3 |
| 28 | 66 | 0 | 38.2 | 38 | 66 | 14.2 | 23.4 |
| 29 | 220 | 0 | 45.1 | 39 | 220 | 20.9 | 34.8 |
| 30 | 220 | 0 | 42.0 | 40 | 220 | 16.5 | 28.0 |

Probably the most striking point shown by Tables II to VI is the gradual increase in the yield of both grain and straw from wheat, oats, and barley and in the hay from rye and timothy. In all cases larger applications of phosphorus gave higher returns, though not always in the same degree.

The grain yield of wheat is especially interesting. Eleven gm. of Tennessee brown rock phosphate produced 1.2 gm. of grain, while double this application produced 4.05 gm., or almost four times the yields from the light-application pots. Pots 7 and 8, which received 6 times as much phosphorus as pots 3 and 4, produced approximately 11 times as much wheat. Pots 9 and 10 received 20 times as much phosphorus as pots 3 and 4, but gave in return only about 14 times as much grain. Scarcely more evidence is necessary to show that wheat is able to take its phosphorus supply from Tennessee brown rock phosphate. It is also evident that the rate of yield is to a certain degree dependent upon the rate of application of the fertilizer. In the case of the heavy application, there were indications that the size of the pot was a limiting factor.

Oats responded more uniformly to the phosphate application than did wheat. The average yield of grain for pots 13 and 14 was 4.6 gm.; pots 15 and 16, which received double the quantity of phosphorus supplied to pots 13 and 14, yielded less than twice the amount of grain. For the highest application there is still a larger difference in the phosphorus applied and the crop produced, due, no doubt, to the limited size of the pot. The yield of straw followed about the same rate of increase as the grain.

Spring rye was not able to endure the heat of the summer days, and at the time of harvest growth had almost ceased without producing a single grain. The hay yield shows a gradual increase in dry matter as the application of phosphate rock was increased.

The yields from barley are not so consistent as those reported for wheat and oats. However, in all probability the same uniformity would have resulted had the crop not been attacked by smut. Although pots 34, 35, 37, 38, and 39 were badly affected, there was a gradual increase in grain and straw as the application of phosphorus increased. A yield of even 18 bushels for barley is not altogether unsatisfactory.

The data on timothy are no less interesting than those on the growth of the cereals, because of the opportunity to study the yields of the various cuttings. Timothy displays the same tendency to produce larger returns for greater quantities of phosphorus applied to the sand. For each pot there was a gradual decrease from the first to the last cutting, although the drop was less abrupt between the first and second than between the third and fourth cuttings.

Contrary to what might be expected the legumes respond to phosphate treatment no better than do the cereals. Perhaps on the whole this latter group produced larger gains than the former.

The results from the cowpeas show some points of particular interest. There was scarcely any seed produced for the pots to which 11 and 22 gm. of raw rock had been applied, but there was a decided increase for the pots which received 66-gm. applications. The next treatment, which was 220 gm. per pot, showed a slight increase, approximately 3 bushels per acre. For the cowpea hay the results are very similar to the seed yield. There is not a very marked increase in the hay production until the larger applications are made. The pots which received 66 gm. produced nearly as much hay as the pots which received 220 gm. of rock phosphate.

Cowpeas do not give results that correspond with those from soybeans. In the first place, the no-treatment pots produced a significant quantity of soy-bean seed, the yield on the acre basis amounting to 2.64 bushels, while the returns from the pots receiving the largest application just about quadrupled those from the former. The ratios for the yields of hay are about the same as for the grain. The yields for both seed and hay in the case of soybeans are unsatisfactory, which is not true of the cowpeas. It would seem that the latter legume utilizes rock phosphate better than soybeans.

To the practical agriculturist the returns from red clover will prove of considerable interest. It will be observed that the lowest treatment, 11 gm. per pot, produced hay at the rate of 772 pounds per acre. With double the application a little less than the former yield is recorded. When the lowest application is increased to six times the original amount, the yield of hay is increased about three times. The largest application, which was 20 times that of the lowest, produced practically 10 times as much hay as the first treatment. The above figures are for the first cutting only.

For the second harvest the relative yields of the 22- and 66-gm. treatments are more satisfactory than for the first cutting. It will be observed that the yield of the pots with 11-gm. applications and those with the 220-gm. applications hold the same relation for the second cutting as for the first. No direct comparison for the third cutting should be made, because pot 79, just previous to cutting, was attacked during a single night by a large cutworm which did considerable damage to the growing crop. It is true, however, that there had not been as much difference in the growth on the high-treatment pot as had been observed earlier in the season. The total yield for three cuttings for the heaviest application is large, but it can hardly be said that the pots which received 22 gm. of rock phosphate produced unprofitable yields.

Because of its extensive root system alfalfa would be expected to produce greater yields than clover. However, the difference in this experiment is not so marked. From four cuttings of alfalfa the yield of hay from the lowest treatment was 5,451 pounds, as against 1,819 pounds

of clover from the same treatment for three cuttings. For the next higher treatment the comparison is 6,426 pounds of alfalfa to 4,674 pounds of clover. The yields are approximately the same for the third application, but for the heavy treatment the clover almost doubles the yield from the alfalfa. Special attention is called to Plates LXXIII, LXXIV, and LXXV.

In drawing conclusions from an investigation of this kind the actual growth of the plant must be regarded as a most significant factor. However, an analytical study of the crops harvested can not fail to be of great value. Since phosphorus is the element with which this paper chiefly concerns itself, quantitative determinations were confined to that substance.

The determinations show that in practically all cases phosphorus is the limiting element in production. In every instance the dry matter increased as the phosphorus content of the pot was increased; also the quantity of phosphorus assimilated increased as the dry matter increased. The percentage of phosphorus in the plant in the majority of cases increased as the application of raw rock grew larger. This is especially noticeable in the hay crop. The most notable exceptions were observed in wheat and oat straw. There is no definite relation in the quantity of phosphorus applied and the percentage assimilated by the crop. There was a slight tendency in the grain for the percentage removed to decrease as the application was increased, but for the legumes this ratio does not hold. As high as 2.49 per cent of the phosphorus supplied in raw rock phosphate was removed in one season's growth of alfalfa.

## COMPARATIVE STUDY OF THE PRODUCTIVE POWERS OF SIX MINERAL PHOSPHATES

The results from Tennessee brown rock phosphate proved so interesting that it was planned to determine the comparative value of mineral phosphates from the various mines of America. For this purpose Tennessee brown rock phosphate, Tennessee blue rock phosphate, South Carolina land rock phosphate, Utah rock phosphate, Canadian apatite, and Florida soft rock phosphate were selected.

The materials were ground so that all particles would pass through a sieve with 100 meshes to the inch and were applied in quantities which contained equal amounts of phosphorus for a given set of pots. Clover, oats, and cowpeas were grown with these different phosphates.

Because of limited space the small battery jars into which could be placed conveniently 4,800 gm. were selected for this rather extensive trial. Without crowding, eight plants per pot could be grown (Pl. LXXVI). Table VII gives the quantity of the phosphate applied and the yields of the crops in question. The planting was done on October 3, 1914, and the crops of clover were harvested on March 5 and April 9, 1915, while the oats were cut on February 5, 1915.

TABLE VII.—*Dry matter produced by different kinds of mineral phosphates—series 2*

| Kind of phosphate added. | Red clover. | | | | Sixty-Day oats. | | |
|---|---|---|---|---|---|---|---|
| | Pot No. | Quantity of phosphate. | First cutting. | Second cutting. | Pot No. | Phosphate added. | Yield of straw. |
| | | *Gm.* | *Gm.* | *Gm.* | | *Gm.* | *Gm.* |
| None | 1 | 0 | 0 | 0 | 39 | 0 | 1.1 |
| Do | 2 | 0 | 0 | 0 | 40 | 0 | 1.0 |
| Tennessee brown rock | 3 | 1.81 | 2.7 | 1.9 | 41 | 1.81 | 4.4 |
| Do | 4 | 1.81 | 1.0 | .5 | 42 | 1.81 | 3.3 |
| Do | 5 | 3.62 | 4.3 | 3.3 | 43 | 3.62 | 5.7 |
| Do | 6 | 3.62 | 4.3 | 3.8 | 44 | 3.62 | 4.2 |
| Do | 7 | 10.86 | 7.1 | 4.8 | 45 | 10.86 | 6.9 |
| Do | 8 | 10.86 | 6.1 | 4.9 | 46 | 10.86 | 6.7 |
| Canadian apatite | 9 | 1.81 | 0 | 0 | 47 | 1.81 | 1.1 |
| Do | 10 | 1.81 | 0 | 0 | 48 | 1.81 | 1.1 |
| Do | 11 | 3.62 | 0 | 0 | 49 | 3.62 | 1.1 |
| Do | 12 | 3.62 | 0 | 0 | 50 | 3.62 | 1.3 |
| Do | 13 | 10.86 | 0 | 0 | 51 | 10.86 | 2.3 |
| Do | 14 | 10.86 | 0 | 0 | 52 | 10.86 | 1.4 |
| South Carolina land rock | 15 | 1.68 | .2 | 1.3 | 53 | 1.68 | 2.2 |
| Do | 16 | 1.68 | 3.3 | 3.8 | 54 | 1.68 | 2.0 |
| Do | 17 | 3.36 | .9 | .8 | 55 | 3.36 | 1.3 |
| Do | 18 | 3.36 | 1.2 | 1.8 | 56 | 3.36 | 2.2 |
| Do | 19 | 10.07 | 1.1 | 0 | 57 | 10.07 | 3.4 |
| Do | 20 | 10.07 | 0 | 0 | 58 | 10.07 | 2.0 |
| Utah rock | 21 | 1.67 | 1.8 | 4.9 | 59 | 1.67 | 2.0 |
| Do | 22 | 1.67 | 3.4 | 5.0 | 60 | 1.67 | 1.4 |
| Do | 23 | 3.34 | 3.3 | 3.0 | 61 | 3.38 | 1.2 |
| Do | 24 | 3.34 | 0 | 0 | 62 | 3.38 | 1.9 |
| Do | 25 | 10.01 | 2.2 | 3.7 | 63 | 10.01 | 1.2 |
| Do | 26 | 10.01 | 2.9 | 4.7 | 64 | 10.01 | 1.5 |
| Tennessee blue rock | 27 | 1.72 | 0 | 0 | 65 | 1.72 | 1.6 |
| Do | 28 | 1.72 | 0 | 0 | 66 | 1.72 | 1.4 |
| Do | 29 | 3.44 | .9 | 1.5 | 67 | 3.44 | 3.0 |
| Do | 30 | 3.44 | .3 | 1.8 | 68 | 3.44 | 3.3 |
| Do | 31 | 10.33 | 7.0 | 6.7 | 69 | 10.33 | 3.3 |
| Do | 32 | 10.33 | 5.6 | 7.6 | 70 | 10.33 | 3.4 |
| Florida soft rock | 33 | 1.65 | 5.0 | 5.1 | 71 | 1.65 | 1.5 |
| Do | 34 | 1.65 | 3.5 | 5.1 | 72 | 1.65 | 1.2 |
| Do | 35 | 3.30 | 6.1 | 5.0 | 73 | 3.30 | 1.9 |
| Do | 36 | 3.30 | 4.4 | 5.9 | 74 | 3.30 | 1.9 |
| Do | 37 | 9.90 | 5.8 | 7.0 | 75 | 9.90 | 3.0 |
| Do | 38 | 9.90 | 5.8 | 6.9 | 76 | 9.90 | 3.2 |

In the foregoing series the greatest contrast is shown by the clover in its response to Tennessee brown rock phosphate and Canadian apatite. With brown rock the yield advanced rapidly with each increase in the amount of phosphate applied; but apatite, even with repeated plantings, failed to produce growth. South Carolina land rock phosphate proved better than apatite, but the growth for this treatment was very irregular. Utah phosphate excelled the South Carolina land rock phosphate. Except for

the lowest treatment, Tennessee blue phosphate gave fairly satisfactory yields. Florida phosphate for the three treatments gave almost as good returns as the Tennessee brown rock. Attention is called to the comparative yields of the Florida rock for the lowest and highest treatments. In this case a smaller quantity of the soft phosphate gave almost as large returns as the greater supply.

TABLE VIII.—*Dry matter produced by different kinds of mineral phosphate in red clover and Sixty-Day oats—series 3*

| Kind of phosphate added. | Red clover. | | | Sixty-Day oats. | | | |
|---|---|---|---|---|---|---|---|
| | Pot No. | Phosphate. | Yield of hay. | Pot No. | Phosphate. | Grain. | Straw. |
| | | *Gm.* | *Gm.* | | *Gm.* | *Gm.* | *Gm.* |
| None | 1 | 0 | 0.1 | 43 | 0 | 0.1 | 1.1 |
| Do. | 2 | 0 | .1 | 44 | 0 | .1 | 1.1 |
| Tennessee brown rock | 3 | 11 | 1.0 | 45 | 11 | .2 | 5.3 |
| Do. | 4 | 11 | 1.0 | 46 | 11 | 1.4 | 5.4 |
| Do. | 5 | 22 | 2.0 | 47 | 22 | 3.2 | 9.5 |
| Do. | 6 | 22 | 2.0 | 48 | 22 | 3.0 | 8.2 |
| Do. | 7 | 66 | 3.9 | 49 | 66 | 5.3 | 10.3 |
| Do. | 8 | 66 | 4.1 | 50 | 66 | 5.2 | 9.0 |
| Do. | 9 | 220 | 3.8 | 51 | 220 | 3.8 | 13.0 |
| Do. | 10 | 220 | 4.0 | 52 | 220 | 5.0 | 12.0 |
| Canadian apatite | 11 | 11 | .1 | 53 | 11 | 0 | 1.4 |
| Do. | 12 | 11 | .1 | 54 | 11 | 0 | 1.4 |
| Do. | 13 | 22 | .2 | 55 | 22 | 0 | 1.4 |
| Do. | 14 | 22 | .2 | 56 | 22 | 0 | 1.6 |
| Do. | 15 | 66 | .1 | 57 | 66 | 0 | 1.3 |
| Do. | 16 | 66 | .3 | 58 | 66 | 0 | 1.0 |
| Do. | 17 | 220 | .1 | 59 | 220 | 0 | 1.2 |
| Do. | 18 | 220 | .1 | 60 | 220 | 0 | 1.5 |
| Utah rock | 19 | 10. 11 | .2 | 61 | 10. 11 | 0 | .8 |
| Do. | 20 | 10. 11 | .2 | 62 | 10. 11 | 0 | .8 |
| Do. | 21 | 20. 22 | .7 | 63 | 20. 22 | 0 | .8 |
| Do. | 22 | 20. 22 | .5 | 64 | 20. 22 | 0 | 1.3 |
| Do. | 23 | 60. 66 | .5 | 65 | 60. 66 | 0 | .9 |
| Do. | 24 | 60. 66 | .4 | 66 | 60. 66 | 0 | .9 |
| Do. | 25 | 202. 20 | .2 | 67 | 202. 20 | 0 | .9 |
| Do. | 26 | 202. 20 | .1 | 68 | 202. 20 | 0 | .9 |
| South Carolina land rock | 27 | 10. 16 | .1 | 69 | 10. 16 | 0 | 1.0 |
| Do. | 28 | 10. 16 | .1 | 70 | 10. 16 | 0 | .8 |
| Do. | 29 | 20. 32 | .1 | 71 | 20. 32 | 0 | .9 |
| Do. | 30 | 20. 32 | .1 | 72 | 20. 32 | 0 | .9 |
| Do. | 31 | 60. 96 | .1 | 73 | 60. 96 | 0 | .7 |
| Do. | 32 | 60. 96 | .1 | 74 | 60. 96 | 0 | .8 |
| Do. | 33 | 203. 20 | .1 | 75 | 203. 20 | 0 | .6 |
| Do. | 34 | 203. 20 | .1 | 76 | 203. 20 | 0 | .5 |
| Tennessee blue rock | 35 | 10. 42 | .2 | 77 | 10. 42 | 0 | .9 |
| Do. | 36 | 10. 42 | .2 | 78 | 10. 42 | 0 | 1.0 |
| Do. | 37 | 20. 84 | .3 | 79 | 20. 84 | 0 | .9 |
| Do. | 38 | 20. 84 | .1 | 80 | 20. 84 | 0 | .7 |
| Do. | 39 | 62. 52 | .4 | 81 | 62. 52 | 0 | .8 |
| Do. | 40 | 62. 52 | .5 | 82 | 62. 52 | 0 | .9 |
| Do. | 41 | 208. 40 | .3 | 83 | 208. 40 | 0 | 1.0 |
| Do. | 42 | 208. 40 | .6 | 84 | 208. 40 | 0 | .9 |

Under greenhouse conditions it was extremely difficult to secure a seed crop of oats during the winter months; hence, the differences of productive power of the various phosphates must be measured by the yields of straw. In a general way the results obtained in this manner are in harmony with those reported for clover. The brown rock excelled the other phosphates in the production of hay; blue phosphate ranks second; and where apatite was applied it will be observed that the plants made very little growth. Plate LXXVI indicates greater difference in the growth of clover than the dry weight of the top.

The above data indicate that there was an increase in yield as the quantity of phosphorus was increased. The question naturally arises as to the point at which larger applications of rock phosphate failed to produce greater returns. In order to answer this query, the following results are inserted (Table VIII): The lowest treatment in the table is about the same as the highest application in series two. This set of pot cultures was planted on August 27, 1914, and harvested on December 4, 1914.

By referring to the clover in Table VIII, a comparison of the yields shows nothing particularly in favor of excessive quantities of rock phosphate. One point, however, is of interest, and that is that the oats produced a seed crop on the land with the heavy application of brown rock. The hay on the other pots was scarcely more than could be produced by the phosphorus in the seeds planted.

TABLE IX.—*Dry matter produced by various kinds of mineral phosphates in cowpeas, series 3*

| Kind of phosphate added. | Pot No. | Phosphate. | Yield of hay. | Kind of phosphate added. | Pot No. | Phosphate. | Yield of hay. |
|---|---|---|---|---|---|---|---|
| | | *Gm.* | *Gm.* | | | *Gm.* | *Gm.* |
| None | 1 | 0 | 1.0 | Utah rock | 22 | 20.22 | 2.2 |
| Do | 2 | 0 | 0.9 | Do | 23 | 60.66 | 1.4 |
| Tennessee brown rock | 3 | 11 | 1.1 | Do | 24 | 60.66 | 1.4 |
| Do | 4 | 11 | 1.9 | Do | 25 | 202.20 | .9 |
| Do | 5 | 22 | 4.6 | Do | 26 | 202.20 | .9 |
| Do | 6 | 22 | 4.5 | | | | |
| Do | 7 | 66 | 11.9 | South Carolina land rock | 27 | 10.16 | 2.0 |
| Do | 8 | 66 | 10.7 | Do | 28 | 10.16 | 2.2 |
| Do | 9 | 220 | 17.4 | Do | 29 | 20.32 | 6.4 |
| Do | 10 | 220 | 13.9 | Do | 30 | 20.32 | 5.4 |
| | | | | Do | 31 | 60.96 | 4.7 |
| Canadian apatite | 11 | 11 | 1.0 | Do | 32 | 60.96 | 4.5 |
| Do | 12 | 11 | 1.0 | Do | 33 | 203.20 | .9 |
| Do | 13 | 22 | 1.0 | Do | 34 | 203.20 | 1.5 |
| Do | 14 | 22 | 1.0 | | | | |
| Do | 15 | 66 | 1.6 | Tennessee blue rock | 35 | 10.42 | 4.0 |
| Do | 16 | 66 | 1.3 | Do | 36 | 10.42 | 3.5 |
| Do | 17 | 220 | .9 | Do | 37 | 20.84 | 4.7 |
| Do | 18 | 220 | .9 | Do | 38 | 20.84 | 5.0 |
| | | | | Do | 39 | 62.52 | 3.9 |
| Utah rock | 19 | 10.11 | 2.0 | Do | 40 | 62.52 | 4.2 |
| Do | 20 | 10.11 | 2.9 | Do | 41 | 208.40 | 1.9 |
| Do | 21 | 20.22 | 3.4 | Do | 42 | 208.40 | 2.1 |

Soon after the clover was harvested in series 3, these pots were seeded to cowpeas. Cowpeas were planted to determine the ability of this legume to utilize the phosphorus contained in mineral phosphates. The cultures were seeded January 24, 1914, and harvested April 5, 1914 (Table IX).

The results secured for series 4 are in accord with those from the clover and oats grown on the pots with large applications. The pots to which had been added brown rock phosphate produced a good return of cowpea hay after having given satisfactory yields of clover.

The data presented in the previous tables show conclusively that certain species of plants have the power to obtain phosphorus from brown rock phosphate, but how they acquire this element is the problem of vital concern. Do they secure their phosphorus without indirect aid and what influence do other plant foods applied in a soluble form exert on the phosphorus compounds?

It will be remembered that the sand cultures were maintained at a moisture content of 14 per cent. The plant food application, the infusion, and the water added when the seeds were planted constituted the first moisture supply; or, in other words, all these solutions brought the water content up to 14 per cent. In most of the cases five applications of plant food were sufficient to produce a crop of clover or oats.

To estimate the influence of water and plant-food solutions on the solubility of the phosphates, quantities of raw rock which correspond to the smallest application (1.81 gm.), soluble plant food equivalent to five applications, and water sufficient to bring the supply of the solution to the same amount that was necessary to bring the moisture content to 14 per cent, or 672 c. c., were placed in a 1-liter flask and shaken each day for three months. The soluble phosphorus was then determined with the results shown in Table X.

TABLE X.—*The influence of soluble plant foods on the solubility of the phosphorus in mineral phosphates*

| Material applied and pot No. | Kind of phosphate. | Quantity of phosphate. | Phosphorus dissolved. |
|---|---|---|---|
| Water only: | | *Gm.* | *Mgm.* |
| 1 | Tennessee brown rock | 1.81 | 0.25 |
| Water and soluble plant food: | | | |
| 2 | do | 1.81 | .33 |
| 3 | Tennessee blue rock | 1.72 | .05 |
| 4 | Canadian apatite | 1.81 | .05 |
| 5 | South Carolina land rock | 1.68 | .056 |
| 6 | Utah rock | 1.67 | .14 |
| 7 | Florida soft rock | 1.65 | .28 |

The solutions dissolve very little of the phosphorus from the insoluble phosphate.

Brown rock phosphate and Florida soft rock phosphate gave the best results with clover, but the former was very much better suited for oats than the latter. There is a slight indication that phosphates which are more soluble in water are more easily assimilated by plants.

## THE INFLUENCE OF FERMENTING DEXTROSE AND CROP RESIDUES ON THE AVAILABILITY OF PHOSPHORUS IN FINELY GROUND ROCK PHOSPHATE

Though the data are not conclusive, a large number of field experiments conducted in America show that raw phosphate, when applied in conjunction with organic matter, produces very appreciable increases in crop yields. The work which follows is an effort to determine the influence of decaying substances on the availability of the phosphorus in crude phosphate rock. Dextrose was employed because it ferments rapidly under greenhouse conditions. Crop residues are also included in this section, but owing to the slow growth of crops through the winter months it will not be possible to do more than to make a preliminary report on this phase of the problem.

Throughout the study included in this division, the glass battery jars were utilized with success and the same quantity of sand employed as previously noted—namely, 4,800 gm. per pot. For all the cultures grown in the dextrose section, the sand was leached with dilute hydrochloric acid.

The first series reported below was outlined primarily to secure data on the value of rock phosphate alone and in conjunction with dextrose for rye and clover. It will be observed that the applications of the rock phosphate and the dextrose were made on the percentage basis. In order to hasten fermentation, an infusion from a rich soil was a part of the treatment. This series was planted on April 12, 1913, and harvested on August 19, 1913.

Since dextrose applied at the rate of 48 gm. per pot injured the rye and destroyed the clover, a point of importance to decide was what quantity would not injure plant development, but would assist in the liberation of phosphorus. With this point in mind, series 6 was planned. The planting was done on June 21, 1913, and the crop harvested on December 1, 1913. (See Table XI.)

The dextrose in series 5 had no beneficial influence. If the average of pots 7, 8, and 9 is compared with the results from either set of pots 1, 2, and 3 or pots 4, 5, and 6, it will be evident that the dextrose is harmful. Clover failed to make growth where the dextrose was added, but did fairly well on the pots which received rock phosphate alone.

The data in Table XI show that dextrose fails to be of any particular advantage for rendering phosphorus available for the growth of rye and clover. Even small quantities of this material killed clover.

TABLE XI.—*Dry matter produced by Tennessee brown rock phosphate and dextrose in growing spring rye and red clover*

SERIES 5

| Rye. | | | | | Red clover. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Pot No. | Phosphate added. | Dextrose added. | Infusion added. | Hay yield. | Pot No. | Phosphate added. | Dextrose added. | Infusion added | Hay yield. |
| | *Gm.* | *Gm.* | *C. c.* | *Gm.* | | *Gm.* | *Gm.* | *C. c.* | *Gm.* |
| 1..... | 48 | 48 | 0 | 22.9 | 19... | 48 | 48 | 20 | [a] 0 |
| 2..... | 48 | 48 | 0 | 33.9 | 20... | 48 | 48 | 20 | [a] 0 |
| 3..... | 48 | 48 | 0 | 31.3 | 21... | 48 | 48 | 20 | [a] 0 |
| 4..... | 48 | 48 | 20 | 35.3 | 22... | 48 | 0 | 0 | 3.0 |
| 5..... | 48 | 48 | 20 | 32.3 | 23... | 48 | 0 | 0 | 3.8 |
| 6..... | 48 | 48 | 20 | 22.0 | 24... | 48 | 0 | 0 | 2.9 |
| 7..... | 48 | 0 | 0 | 27.2 | | | | | |
| 8..... | 48 | 0 | 0 | 32.5 | | | | | |
| 9..... | 48 | 0 | 0 | 36.0 | | | | | |

SERIES 6

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 3.... | 48 | 4.8 | 20 | 14.6 | 17... | 48 | 4.8 | 20 | [b] 20 |
| 4.... | 48 | 4.8 | 20 | 17.9 | 18... | 48 | 4.8 | 20 | [b] 20 |
| 5.... | 48 | 14.4 | 20 | 17.5 | 19... | 48 | 14.4 | 20 | [b] 20 |
| 6.... | 48 | 14.4 | 20 | 16.0 | 20... | 48 | 14.4 | 20 | [b] 20 |
| 7.... | 48 | 24.0 | 20 | 16.8 | 21... | 48 | 24.0 | 20 | [b] 20 |
| 8.... | 48 | 24.0 | 20 | 20.7 | 22... | 48 | 24.0 | 20 | [b] 20 |
| 9.... | 48 | 48.0 | 20 | 14.5 | 23... | 48 | 48.0 | 20 | [b] 20 |
| 10.... | 48 | 48.0 | 20 | 11.6 | 24... | 48 | 48.0 | 20 | [b] 20 |
| 11.... | 48 | 0 | 20 | 18.7 | 25... | 48 | 0 | 20 | 3.0 |
| 12.... | 48 | 0 | 20 | 17.7 | 26... | 48 | 0 | 20 | 2.9 |
| | | | | | 27... | 0 | 0 | 20 | 0 |
| | | | | | 28... | 0 | 0 | 20 | 0 |

[a] The clover in pots 19, 20, and 21 was dead on June 29, 1913.
[b] The clover in pots 17 to 24, inclusive, was dead in less than 1 month after planting.

Rye and clover were replaced in series 7 (Table XII) by cowpeas, with the feeling that the latter crop might respond more readily to various treatments (Pl. LXXVII). The cowpeas were planted on July 4, 1913, and harvested on October 2, 1913.

The cowpeas grown in series 7 show clearly that so small a quantity of dextrose as 4.8 per cent was injurious to plant growth. Where dextrose was applied, smaller quantities of phosphorus were assimilated, due, no doubt, to the injury of the plant by the acids formed from decomposing dextrose. However, the percentage of phosphorus increased as the quantity of the fermentable substance was increased.

TABLE XII.—*Dry matter and phosphorus content of plant products of cowpeas from pot cultures, with the addition of Tennessee brown rock phosphate and dextrose; series 7*

| Pot No. | Phosphate added. | Dextrose added. | Infusion added. | Hay yield. | Phosphorus content. | | |
|---|---|---|---|---|---|---|---|
| | | | | | Hay. | Hay yield. | Removed from pot. |
| | *Gm.* | *Gm.* | *C. c.* | *Gm.* | *Per cent.* | *Mgm.* | *Per cent.* |
| 1..... | 48 | 4.8 | 20 | 25.0 | 0.319 | 79.75 | 1.30 |
| 2..... | 48 | 4.8 | 20 | 22.3 | ..... | ..... | ..... |
| 3..... | 48 | 14.4 | 20 | 11.9 | ..... | ..... | ..... |
| 4..... | 48 | 14.4 | 20 | 8.8 | .381 | 33.33 | .54 |
| 5..... | 48 | 24.0 | 20 | [a] 0 | ..... | ..... | ..... |
| 6..... | 48 | 24.0 | 20 | [a] 0 | ..... | ..... | ..... |
| 7..... | 48 | 48.0 | 20 | [a] 0 | ..... | ..... | ..... |
| 8..... | 48 | 48.0 | 20 | [a] 0 | ..... | ..... | ..... |
| 9..... | 0 | 48.0 | 20 | [a] 0 | ..... | ..... | ..... |
| 10..... | 0 | 48.0 | 20 | [a] 0 | ..... | ..... | ..... |
| 11..... | 48 | 0 | 20 | 29.9 | ..... | ..... | ..... |
| 12..... | 48 | 0 | 20 | 29.1 | .286 | 85.51 | 1.40 |
| 13..... | 0 | 0 | 20 | 4.0 | ..... | ..... | ..... |
| 14..... | 0 | 0 | 20 | 3.0 | [b].128 | 3.84 | ..... |

[a] The plants on pots 5 to 10, inclusive, were all dead by Aug. 9, 1913.
[b] Five cowpea seeds were planted in each pot. These contained 3.92 mgm. of phosphorus.

Rye, clover, and cowpeas failed to thrive wherever the smallest quantity of dextrose was present. There is but little doubt that this destructive influence is due to the decomposition of dextrose. If this conclusion be true, a liberal use of calcium carbonate should neutralize the acids developed, and a normal growth of the plants should result. Series 8 (Table XIII) was designed for determining what influence calcium carbonate would have in stimulating plant growth by producing an alkaline medium and to ascertain whether calcium served as a food.

TABLE XIII.—*Dry matter produced in spring rye by Tennessee brown rock phosphate with the addition of dextrose and calcium carbonate—series 8*

| Pot No. | Phosphate added. | Dextrose added.[a] | Calcium carbonate added. | Hay yield. | Pot No. | Phosphate added. | Dextrose added.[a] | Calcium carbonate added. | Hay yield. |
|---|---|---|---|---|---|---|---|---|---|
| | *Gm.* | *Gm.* | *Gm.* | *Gm.* | | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1.... | 48 | 48 | 10 | 7.2 | 13... | 0 | 0 | 0 | 0.5 |
| 2.... | 48 | 48 | 10 | 9.3 | 14... | 0 | 0 | 0 | .6 |
| 3.... | 48 | 48 | 0 | 5.2 | 15... | 48 | 0 | 0 | 11.2 |
| 4.... | 48 | 48 | 0 | 3.5 | 16... | 48 | 0 | 0 | 12.1 |
| 5.... | 48 | 48 | 10 | 7.0 | 17... | 48 | 4.8 | 10 | 10.7 |
| 6.... | 48 | 48 | 10 | 7.0 | 18... | 48 | 4.8 | 10 | 10.0 |
| 7.... | 0 | 0 | 0 | .1 | 19... | 48 | 4.8 | 0 | 9.6 |
| 8.... | 0 | 0 | 0 | .1 | 20... | 48 | 4.8 | 0 | 9.0 |
| 9.... | 48 | 48 | 0 | 7.8 | 21... | 0 | 0 | 0 | .4 |
| 10.... | 48 | 48 | 0 | 8.9 | 22... | 0 | 0 | 0 | .3 |
| 11.... | 0 | 0 | 0 | .1 | 23... | 48 | 48 | 0 | 6.5 |
| 12.... | 0 | 0 | 0 | .1 | 24... | 48 | 48 | 0 | 6.0 |

[a] Pots 5 and 6 were leached and the leachings placed on pots 7 and 8. Pots 9 and 10 were leached and the leachings placed on pots 11 and 12. Pots 13 and 14 received all plant food but phosphorus. Pots 21 and 22 received nothing. Pots 23 and 24 were leached and drainage water taken for analytical purposes.

Series 8 shows that dextrose in conjunction with calcium carbonate did not give as good results as raw rock phosphate alone, and that 10 gm. of calcium carbonate was not sufficient to nullify the harmful influence of the dextrose.

Series 9 (Table XIV), wnich follows, is just the same as series 8 except that cowpeas are substituted for rye, the object being to determine the relative response of rye and cowpeas to the different treatments.

TABLE XIV.—*Dry matter produced in cowpeas by Tennessee brown rock phosphate with the addition of dextrose and calcium carbonate—series 9*

| Pot No. | Phosphate added. | Dextrose added. | Calcium carbonate added. | Hay yield. | Pot No. | Phosphate added. | Dextrose added. | Calcium carbonate added. | Hay yield. |
|---|---|---|---|---|---|---|---|---|---|
| | *Gm.* | *Gm.* | *Gm.* | *Gm.* | | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1F.. | 48 | 48 | 10 | 5.5 | 13F.. | 0 | 0 | 0 | 3.3 |
| 2F.. | 48 | 48 | 10 | 5.3 | 14F.. | 0 | 0 | 0 | 3.0 |
| 3F.. | 48 | 48 | 0 | 5.2 | 15F.. | 48 | 0 | 0 | 12.4 |
| 4F.. | 48 | 48 | 0 | 4.0 | 16F.. | 48 | 0 | 0 | 14.5 |
| 5F.. | 48 | [a] 48 | 10 | 5.3 | 17F.. | 48 | 4.8 | 10 | 8.1 |
| 6F.. | 48 | 48 | 10 | 6.1 | 18F.. | 48 | 4.8 | 10 | 10.1 |
| 7F.. | 0 | 0 | 0 | 3.8 | 19F.. | 48 | 4.8 | 0 | 11.1 |
| 8F.. | 0 | 0 | 0 | 3.8 | 20F.. | 48 | 4.8 | 0 | 11.8 |
| 9F.. | 48 | 48 | 0 | 4.2 | 21F.. | 0 | 0 | 0 | 3.9 |
| 10F.. | 48 | 48 | 0 | 3.0 | 22F.. | 0 | 0 | 0 | 4.5 |
| 11F.. | 0 | 0 | 0 | 3.1 | 23F.. | 48 | 48 | 0 | 4.0 |
| 12F.. | 0 | 0 | 0 | 2.3 | 24F.. | 48 | 48 | 0 | 4.0 |

[a] See note to Table XIII.

Series 9 shows that brown rock phosphate, dextrose, and a limited supply of calcium carbonate failed to give as good results with cowpeas as raw phosphate alone. For further comparison see Plate LXXVIII.

Thus far it has not seemed necessary to use calcium carbonate alone, because it was thought that the plants would get enough calcium, for full growth from the phosphate, however, in order to avoid criticism at this point calcium carbonate was added to certain pots in the following series. The quantity of this compound was increased to 48 gm. per pot, which is almost five times as much as the application in the preceding series.

By making a comparison of the pots which received raw rock phosphate alone and those which received raw rock and calcium carbonate very little difference in the yield is observed, only 0.6 gm. more in favor of the addition of the lime compound. There is no strong evidence in Table XV to show that the omission of calcium was a mistake. Where lime was applied with rock phosphate and dextrose, the injury by dextrose reported earlier was nullified by the application of lime (Pl. LXXIX and LXXX).

Attention is called to the percentage of phosphorus in the cowpea hay grown in the pots which received soluble phosphorus.

TABLE XV.—*Dry matter produced in cowpeas by Tennessee brown rock phosphate with the addition of dextrose and calcium carbonate—series 10*

| Pot No. | Phosphate added. | Dextrose added. | Calcium added. | Hay yield. | Phosphate content. | | |
|---|---|---|---|---|---|---|---|
| | | | | | Hay. | | Percentage removed from pot. |
| | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Per cent.* | *Mgm.* | |
| 1G... | 0 | 0 | 10 | 3.0 | 0.111 | 3.37 | ..... |
| 2G... | 0 | 0 | 10 | 3.5 | ..... | ..... | ..... |
| 3G... | 0 | 0 | 0 | 2.8 | ..... | ..... | ..... |
| 4G... | 0 | 0 | 0 | 3.0 | .119 | 3.57 | ..... |
| 5G... | 48 | 0 | 0 | 6.1 | ..... | ..... | ..... |
| 6G... | 48 | 0 | 0 | 6.3 | .246 | 15.55 | 0.25 |
| 7G... | 48 | 0 | 0 | 5.3 | ..... | ..... | ..... |
| 8G... | 48 | 0 | 0 | 6.8 | .236 | 16.05 | .52 |
| 9G... | 48 | 0 | 10 | 7.4 | .156 | 11.52 | .19 |
| 10G... | 48 | 0 | 10 | 6.2 | .239 | 11.71 | .19 |
| 11G... | 48 | 48 | 0 | 4.9 | ..... | ..... | ..... |
| 12G... | 48 | 48 | 0 | 4.6 | ..... | ..... | ..... |
| 13G... | 0 | 48 | 48 | 3.2 | .054 | 1.73 | ..... |
| 14G... | 0 | 48 | 48 | 3.3 | ..... | ..... | ..... |
| 15G... | 48 | 48 | 10 | 6.8 | ..... | ..... | ..... |
| 16G... | 48 | 48 | 10 | 7.0 | .122 | 8.54 | .14 |
| 17G... | (a) | 0 | 0 | 4.9 | ..... | ..... | ..... |
| 18G... | (a) | 0 | 0 | 4.8 | .660 | 31.68 | ..... |
| 19G... | 0 | 0 | 0 | 3.0 | ..... | ..... | ..... |
| 20G... | 0 | 0 | 0 | 3.0 | .104 | 5.19 | ..... |
| 21G... | b 48 | 0 | 0 | 7.0 | ..... | ..... | ..... |
| 22G... | 48 | 0 | 0 | 6.8 | .388 | 24.06 | .39 |
| 23G... | 48 | 48 | 48 | 6.9 | ..... | ..... | ..... |
| 24G... | 48 | 48 | 48 | 6.3 | .115 | 7.25 | .12 |

*a* Soluble phosphate.
*b* In pots 21 and 22 potassium chlorid was substituted for potassium sulphate.

Under the conditions of this experiment, fermenting dextrose was a failure in bringing about the liberation of phosphorus. Since the use of crop residues is a common farm practice for supplying organic matter, which is said to aid in the liberation of phosphorus, the series next reported was planned with timothy hay and clover substitutes for dextrose.

Timothy and clover cultures on which data are reported in Table III are used for this phase of the problem. Of the duplicate pots the hay from one was taken for analytical study, while the product of the other was ground and returned as organic matter. This series (Table XVI) shows the original treatment with the quantity of air-dried hay turned under. The contents of the pots to which organic matter was added were turned out and the ground material thoroughly incorporated with the sand on December 3, 1914. On January 23, 1915, the pots were planted to the respective crops. They were harvested on April 17, 1915.

The organic matter with phosphate in the above series gave larger returns in most cases than where the phosphate was alone. This increase is probably due to the liberation of phosphorus by the decaying residues or the organic phosphorus in the crop residues themselves.

TABLE XVI.—*Dry matter produced in timothy and red clover by Tennessee brown rock phosphate and crop residues—series 11*

| Timothy. | | | | Red clover. | | | |
|---|---|---|---|---|---|---|---|
| Pot No. | Phosphate added. | Organic matter added. | Hay yield. | Pot No. | Phosphate added. | Organic matter added. | Hay yield. |
| | *Gm.* | *Gm.* | *Gm.* | | *Gm.* | *Gm.* | *Gm.* |
| 41[a] | 0 | 0 | 0.25 | 71 | 0 | 0 | 0.02 |
| 42 | 0 | 2.9 | .05 | 72 | 0 | .55 | .10 |
| 43 | 11 | 0 | .02 | 73 | 11 | 0 | .40 |
| 44 | 11 | 24.4 | .20 | 74 | 11 | 10.5 | .10 |
| 45 | 22 | 0 | .30 | 75 | 22 | 0 | 2.70 |
| 46 | 22 | 32.7 | 1.10 | 76 | 22 | 30.5 | 6.40 |
| 47 | 66 | 0 | 3.40 | 77 | 66 | 0 | 4.70 |
| 48 | 66 | 61.6 | 10.70 | 78 | 66 | 57.9 | 12.50 |
| 49 | 220 | 0 | 8.70 | | | | |
| 50 | 220 | 67.2 | 11.70 | | | | |

[a] See series 1, Tables II to VI.

## INFLUENCE OF SIZE OF PARTICLES ON THE AVAILABILITY OF PHOSPHORUS IN MINERAL PHOSPHATES

The degree of fineness of rock phosphate particles has been held by many investigators to be an important factor in the availability of mineral phosphates. Dr. Jordan, of the New York Experiment Station, showed rather conclusively that plants supplied with very finely ground rock phosphate contained more phosphorus and produced a greater quantity of dry matter than those supplied with the coarser grades. For the purpose of determining a comparative value of the same rock when ground very fine to that left in particles of a larger size, series 12 (Table XVII) was begun. As a check on the rock which was obtained from the Mount Pleasant mills some lump rock from the same source was secured and ground. These results are reported along with the data on the influence of the size of particles on the availability.

TABLE XVII.—*Relation of size of phosphate particles to the availability of phosphorus by Sixty-Day oats harvested on July 10, 1915—series 12*

| Pot No. | Phosphate added. | Fineness. | Grain yield. | Straw yield. | Pot No. | Phosphate added. | Fineness. | Grain yield. | Straw yield. |
|---|---|---|---|---|---|---|---|---|---|
| | *Gm.* | | *Gm.* | *Gm.* | | *Gm.* | | *Gm.* | *Gm.* |
| 1 | 0 | ..... | 0 | 1.20 | 10 | 2.6 | 200 degrees or over..... | 4.00 | 6.3 |
| 2 | 0 | ..... | 0 | 1.60 | 17 | 2.6 | 80 to 100 degrees..... | 5.90 | 6.80 |
| 5 | 2.6 | 80 to 100 degrees..... | 3.25 | 6.15 | 18 | 2.6 | .....do..... | 7.00 | 10.05 |
| 6 | 2.6 | .....do..... | 2.05 | 5.35 | 19 | 2.6 | 100 to 200 degrees..... | 5.80 | 11.05 |
| 7 | 2.6 | 100 to 200 degrees..... | 3.70 | 6.60 | 20 | 2.6 | .....do..... | 7.70 | 11.35 |
| 8 | 2.6 | .....do..... | 4.70 | 9.30 | 21 | 2.6 | 200 degrees or over..... | 8.65 | 11.10 |
| 9 | 2.6 | 200 degrees or over..... | 5.00 | 8.70 | 22 | 2.6 | .....do..... | 7.15 | 13.20 |

Pots 5 to 10, inclusive, received the ground rock phosphate as it was obtained from the mills. The degree of fineness varied from that passing a sieve 80 to 100 meshes to the inch to that which would go through a sieve of 200 meshes to the inch. Pots 17 to 22, inclusive, received the ground phosphate which was shipped in the lump form and afterward ground to the same degree of fineness as that ground at the mill.

There is a tendency for the dry matter to increase as the degree of fineness increases. The phosphate received from the mill in lump form was slightly better than that sent to us in a ground condition.

## DISCUSSION

Under the conditions of these experiments a fairly large portion of the phosphorus in brown rock phosphate was available for plant growth. The quantity was variable, depending upon the crops and the circumstances attending the full development of the plant. The data show only a very small amount of phosphorus soluble in water and plant food solutions. It is clear that other factors which might bring about availability must be considered. The sand cultures contained very little organic matter; hence, these slight fermentable substances should not be considered. There is nothing left but the plant for our examination and there is abundant proof that the plant itself is a significant item. Since plants excrete large quantities of carbonic acid, there is but little question that this substance plays the primary roll in the liberation of phosphorus.

The reactions with carbon dioxid which occur when tricalcium phosphate is put into sand cultures of the kind described in these pages may be shown in the following manner:

$$\underset{\text{Gaseous}}{CO_2 + H_2O \rightleftarrows H_2CO_3} \rightleftarrows \underset{\text{In solution}}{H^+ + \overline{HCO_3}} \rightleftarrows H^{++} + CO_3 \quad \text{(A)}$$

(1) (2) (3)

$$\underset{\text{Solid}}{Ca_3(PO_4)_2} \rightleftarrows Ca_3(PO_4)_2 \rightleftarrows \underset{\text{In solution.}}{3Ca^{++} + \overline{\overline{2PO_4}}} \quad \text{(B)}$$

(4) (5)

When A and B are mixed, the following equilibria develop:

$$\overline{\overline{PO_4}} + H^+ \overline{HPO_4} \quad \text{(C) (The ion of reverted phosphate)}$$

(6)

$$\overline{\overline{HPO_4}} + H^+ \rightleftarrows H_2PO_4 \text{ (The ion of soluble phosphate)}$$

(7)

$$\overline{H_2PO_4} + H^+ \rightleftarrows H_3PO_4$$

(8)

Equations A and B make it evident that the hydrogen ion concentration for the various acids will determine the course of the reactions rendering the rock phosphate available. The hydrogen ion concentration is made up of two factors—namely, the concentration and the strength of the acid. Obviously under the conditions of these experiments saturated solutions of rock phosphate and carbonic acid are employed. The relative insolubility of the rock phosphate tends to decrease greatly the concentration of the $H^+$ from either 6, 7, or 8. The relatively greater solubility of the calcium bicarbonate, since it furnishes $\overline{HCO_3}$, would also tend to decrease the $H^+$ concentration from carbonic acid, but this factor of common ion effect is of far less importance upon the concentration of the $H^+$ from $H_2CO_3$ than the solubility of the tricalcium phosphate upon equations 6, 7, and 8, especially since the $Ca^{++}$ from the $Ca(HCO_3)_2$ is removed by plants.

Assuming equivalent or unit concentrations of the substances $H_2CO_3$, $H_3PO_4$, $\overline{H_2PO_4}$, and $\overline{\overline{HPO_4}}$ are present—that is, eliminating the factor of concentration of the substances producing the H—the relative strength of these acids is given by their ionization constants, thus:

(1)[a] $H_2CO_3 \rightleftarrows H^+ + \overline{HCO_3}$ $\quad Ka^{18°}\ 3.0\times10^{-7}$

(6) $\overline{\overline{HPO_4}} \rightleftarrows \overline{\overline{\overline{PO_4}}} + H^+$ $\quad Ka^{18°}\ 3.6\times10^{-13}$

(7) $\overline{H_2PO_4} \rightleftarrows \overline{\overline{HPO_4}} + H^+$ $\quad Ka^{18°}\ 1.95\times10^{-7}$

(8) $H_3PO_4 \rightleftarrows \overline{H_2PO_4} + H^+$ $\quad Ka^{18°}\ 1.1\times10^{-2}$

The mass law for monobasic acids (HAc) has the form $Ka = \frac{(\text{Conc } H^+)\ (\text{Conc Ac})}{\text{Conc HAc}}$. Since the acids of equations 1, 6, and 7 are weak acids ($Ka < 10^{-4}$), the mass law assumes the form $Ka = (\text{Conc } H^+)\ (\text{Conc Ac}) = (\text{Conc } H^+)^2$, because the concentration of HAc is practically unity. The concentrations of $H^+$ for these equations at 18° C. are for:

(1) $\sqrt{3\times10^{-7}} = 5.5\times10^{-4}$ for $C^{(1)}H^+$

(6) $\sqrt{3.6\times10^{-13}} = 6\times10^{-7}$ for $C^{(6)}H^+$

(7) $\sqrt{1.95\times10^{-7}} = 4.4\times10^{-4}$ for $C^{(7)}H^+$

For the first hydrogen of $H_3PO_4$ the above expression can not be used, since the amount $H_3PO_4$ compared to its ions is small rather than large. Here the mass law must be used in its true form, $K = \frac{C\alpha^2}{1-\alpha}$, where C is equal to the initial concentration of $H_3PO_4$ and $\alpha$ degrees of ioniza-

[a] See equations, page 508.

tion. For the purpose $\alpha$ is taken equal to 90 per cent, from which the concentration of H is calculated thus:

$$Ka^{18°} = \frac{C\alpha^2}{1-\alpha}, \quad \text{where} \quad (C) = Ka\frac{(1-\alpha)}{\alpha} = \text{concentration of H.}$$

$$\therefore \text{ concentration of } H^+(C^{(8)}H^+) = \frac{1.1 \times 10^{-2}(0.1)}{0.9} = \frac{1.1 \times 10^{-3}}{0.9} = 1 \times 10^{-3} = 0.001.$$

It is seen that only the first H of $H_3PO_4$ can furnish a greater concentration of $H^+$ than $H_2CO_3$ for equivalent concentrations. In the actual experiment the concentration of $H_3PO_4$ is much less than that of $H_2CO_3$. However, the availability of the rock phosphate by means of $H_2CO_3$ is not conditioned by the liberation of free $H_3PO_4$ according to equation 8. Equation 6 or 7 is driven in the direction to remove $H^+$, would render the tricalcium phosphate more available, but a reaction between ions proceeds if a lesser ionized product be formed. Calculations of the $H^+$ concentration for equations 1, 6, and 7 shows that for equivalent concentrations the $H^+$ from carbonic acid is greatly in excess of the $H^+$ concentration for equations 6 and 7. So if equations 1, 6, and 7 are present simultaneously $\overline{\overline{HPO_4}}$ and $\overline{H_2PO_4}$ of equations 6 and 7 would be formed by the union of $H^+$ of $H_2CO_3$ with $\overline{\overline{PO_4}}$ and $\overline{\overline{HPO_4}}$, respectively, thus causing more $Ca_3(PO_4)_2$ to dissolve to reestablish the equilibria for equations 6 and 7. It is a fact, however, that a greater concentration of $H_2CO_3$ is present than any of the ionizing substances, as $\overline{\overline{HPO_4}}$, $\overline{H_2PO_4}$, or $H_3PO_4$. This would increase the rate of availability of the tricalcium phosphate.

These calculations are borne out by the fact that more $Ca_3(PO_4)_2$ dissolved in water containing $H_2CO_3$ than in pure water. Seidel's solubility tables state that 1 liter of water saturated with $H_2CO_3$ dissolves 0.15 to 0.30 gm. of $Ca_3(PO_4)_2$ at 25°, while 1 liter of pure water dissolved only 0.01 to 0.10 gm. of $Ca_3(PO_4)_2$ at 25°.

Reactions 6 and 7 may be shown in the nonionic form as follows:

$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows Ca_2H_2(PO_4)_2 + CaH_2(CO_3)_2 \text{ or}$$
$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows Ca_2(HPO_4(_2 + Ca(HCO_3)_2$$
$$Ca_2H_2(PO_4)_2 + 2H_2CO_3 \rightleftarrows CaH_4(PO_4)_2 + CaH_2(CO_3)_2$$
$$\downarrow \qquad\qquad \downarrow$$
$$\text{or} \qquad\qquad \text{or}$$
$$Ca(H_2PO_4)_2 \qquad Ca(HCO_3)_2$$

In the first equation calcium is found in a form readily assimilated by plants, and in the second the monocalcium phosphate is in a very assimilable form. On this equation we have based our belief that there is no necessity for applying lime to sand cultures to which had previously been

added raw rock phosphate. When the calcium bicarbonate and monocalcium phosphate are both removed from the medium of growth by plants, the reaction is driven rapidly to the right. Mass relationship in a mixture of this kind confirms such an interpretation as the one presented above. Our first assumption, that plants should get their calcium from rock phosphate in the same manner that they get their phosphorus, is supported at several points in this work. This must be so, since the calcium is furnished by the calcium salt of phosphoric acid or by the bicarbonate. There was no greater growth when calcium carbonate was added than where raw rock alone was used. In fact, the growth might be even less, since calcium carbonate might furnish a greater concentration of $Ca(HCO_3)_2$ or $HCO_3$, which might decrease the concentration of H from equation 1, thus decreasing the rate of the availability of rock phosphate.

The most marked feature of the investigation is the difference of the availability of the various minerals. The fact that the crop yields increase as the application of the brown rock phosphate was increased indicates that a portion of the phosphorus was readily assimilated while the plants were young, and that by the time these plants became well established they were able to utilize the more insoluble form. If we are to assume that a part of the phosphorus is of animal origin, this position probably is more tenable, or on the other hand, through long years of weathering the compound had been so changed that a portion was more easily taken up by plants than before weathering began.

There is an indication that the crops grown first took up the more available phosphorus and that the second crop made very slow growth because the more soluble phosphorus was removed by the first crop and nothing left but the rather insoluble for later crops. These points have proof from the cowpeas on the large application series and the clover on the crop residue series.

Brown rock phosphate and Florida soft rock phosphate lead the others in supplying available phosphorus for plant nutrition, especially for clover. The brown rock phosphate leads for all the crops. These two phosphates gave the largest quantity of phosphorus soluble in water and plant-food solutions. The results indicate a relation in solubility in plant-food solution and the availability for plants.

The difference in the assimilation of these phosphates can not be attributed to the degree of fineness of the particles, since they were all ground, so that the entire sample passed through a sieve of 100 meshes to the inch. If the degree of fineness influenced the results, the differences then come from the size of particles, which were smaller than those found in commercial phosphates.

The variation in the agricultural value of the six mineral phosphates studied is difficult to explain. Their productive powers seemed not to

have any direct relation to the amount of phosphorus which they contained. Brown rock, which had the smallest amount of phosphorus, produced the most satisfactory yields. The differences must be attributed to modes of formation and weathering since the minerals were laid down.

## SUMMARY

(1) Phosphorus in rock phosphate can be assimilated by farm crops in sand cultures under greenhouse conditions, even in the absence of decaying residues.

(2) Crop residues, when employed in conjunction with brown rock phosphates, were beneficial.

(3) Tennessee brown rock phosphate, Florida soft rock phosphate, and Tennessee blue rock phosphate in the heavier applications proved superior to South Carolina land rock phosphate, Utah rock phosphate, and Canadian apatite, for oats, clover, and cowpeas when grown in sand.

(4) The phosphorus in brown rock phosphate and Florida soft rock phosphate was more soluble in water and in plant-food solutions than the phosphorus in other mineral phosphates. The superiority of these two phosphates over the others tested is shown chiefly by the first crop.

(5) Chemical analysis showed that the plant-food solutions applied did not appreciably modify the results.

(6) The cereals produced as satisfactory yields as the legumes.

(7) The crop yields tended to increase as the application of rock phosphate increased up to a point where the size of the pots seemed to be a limiting factor, apatite being the only exception.

(8) The plants obtained their calcium, as well as their phosphorus, from brown rock phosphates. No better results were secured when calcium carbonate was applied than when rock phosphate alone was used.

(9) There was no particular relation between the citric-acid-soluble phosphorus and the availability of these phosphates for plants.

(10) Dextrose, when used as a fermentable substance, was harmful.

(11) The degree of fineness is a factor which determines to some extent the availability of rock phosphate, as indicated by the brown rock.

(12) These investigations extended over a period of 3½ years, and embrace results from 700 pot cultures and 400 phosphorus determinations.

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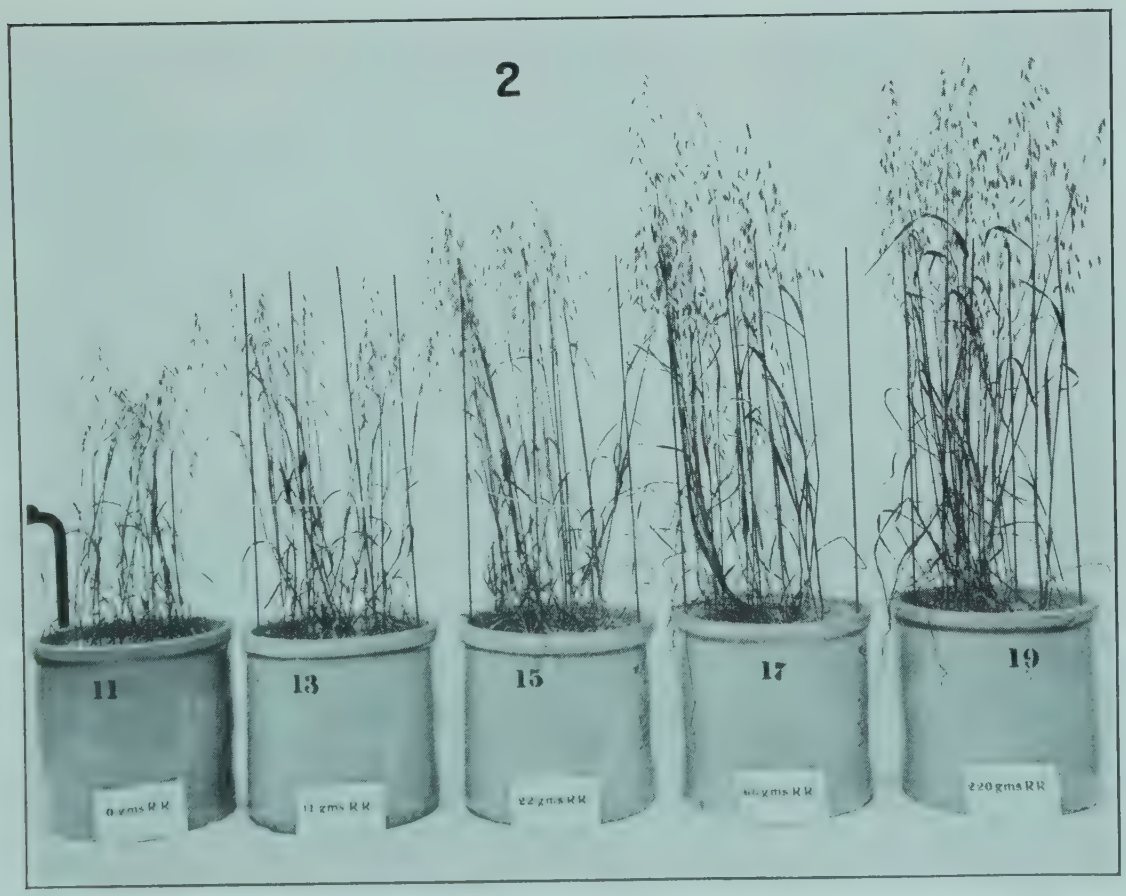
## PLATE LXXIII

Effect of varying quantities of Tennessee brown rock phosphates on plant growth:

Fig. 1.—Spring wheat. (Table II, Series 1A.)
Fig. 2.—Sixty-Day oats. (Table II, series 1B.)

1
1
3
5
7
9


2
11
13
15
17
19

1
31
33
35
37
39

2
41
43
45
47
49

## PLATE LXXIV

Effect of varying quantities of Tennessee brown rock phosphate on plant growth:

Fig. 1.—Barley. (Table VI.)
Fig. 2.—Timothy. (Table III, series 1E.)

37770°—16——4

## PLATE LXXV

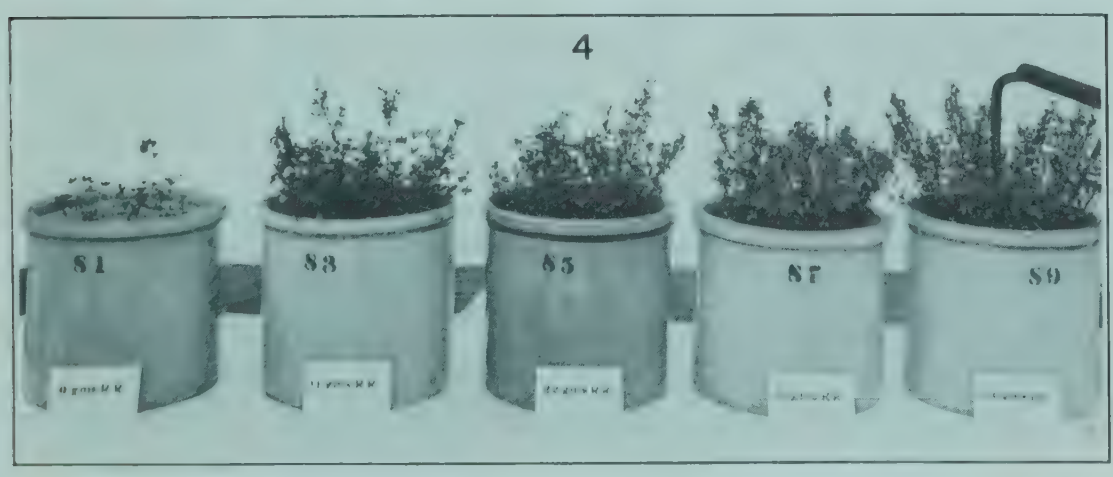
Effect of varying quantities of Tennessee brown rock phosphate on plant growth;

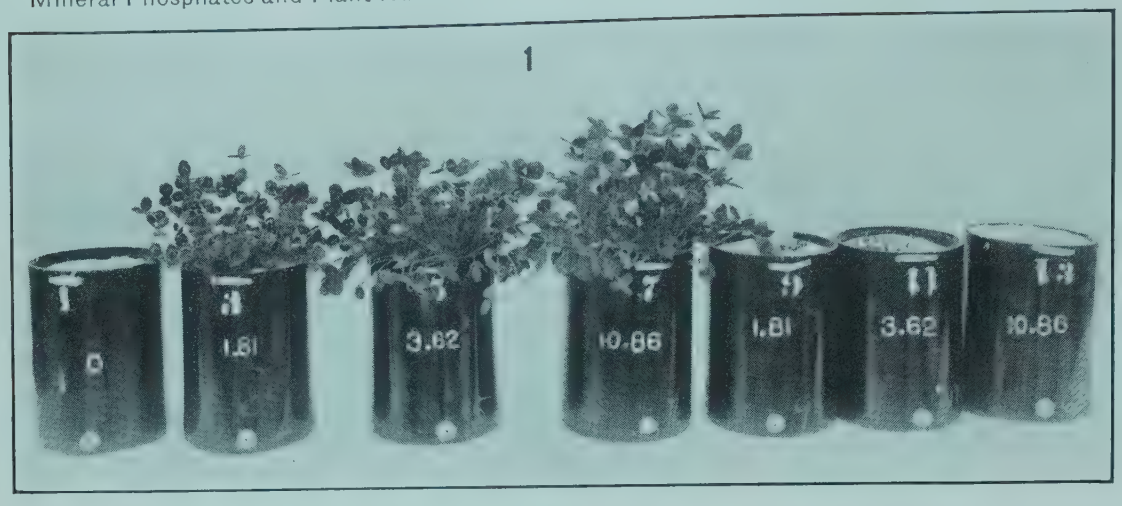
Fig. 1.—Cowpeas. (Table IV, series 1F.)
Fig. 2.—Soybeans. (Table IV, series 1G.) Photographed just before cutting.
Fig. 3.—Red clover. (Table III, series 1H.)
Fig. 4.—Alfalfa. (Table V.) Photographed before first cutting.

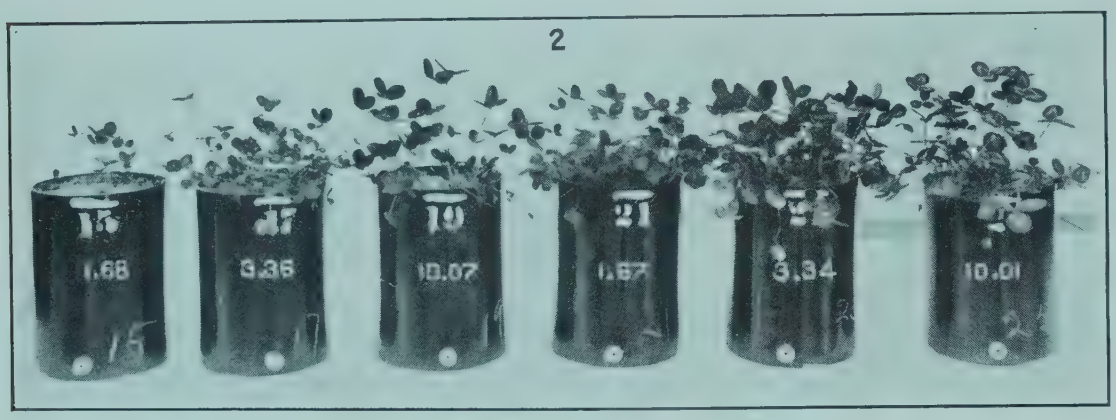
1
51
53
55
57
59

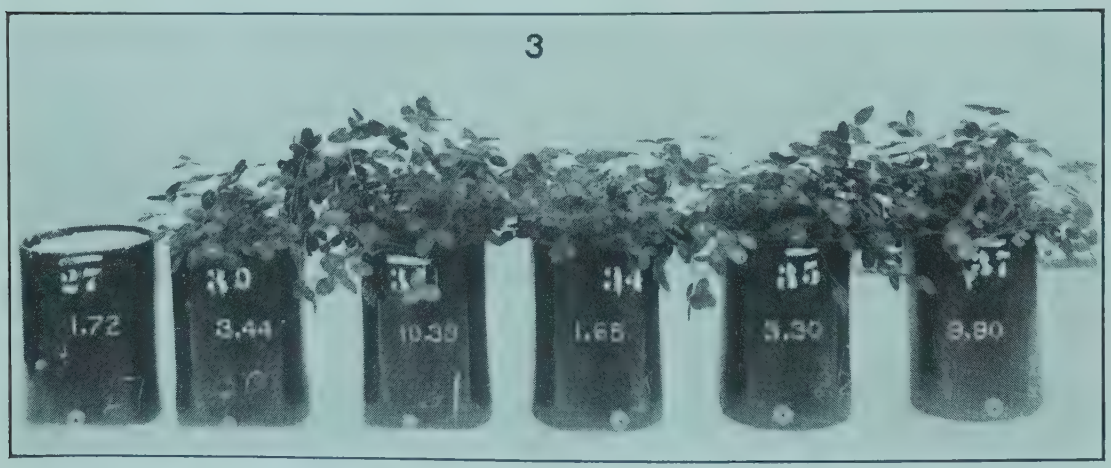
2
61
63
65
67
69

3
71
73
75
77
79


4
81
83
85
87
89


1
1
0
3
1.81
5
3.62
7
10.86
9
1.81
11
3.62
13
10.86


2
15
1.68
17
3.36
19
10.07
21
1.67
23
3.34
25
10.01


3
27
1.72
29
3.44
31
10.39
33
1.66
35
3.30
37
9.80

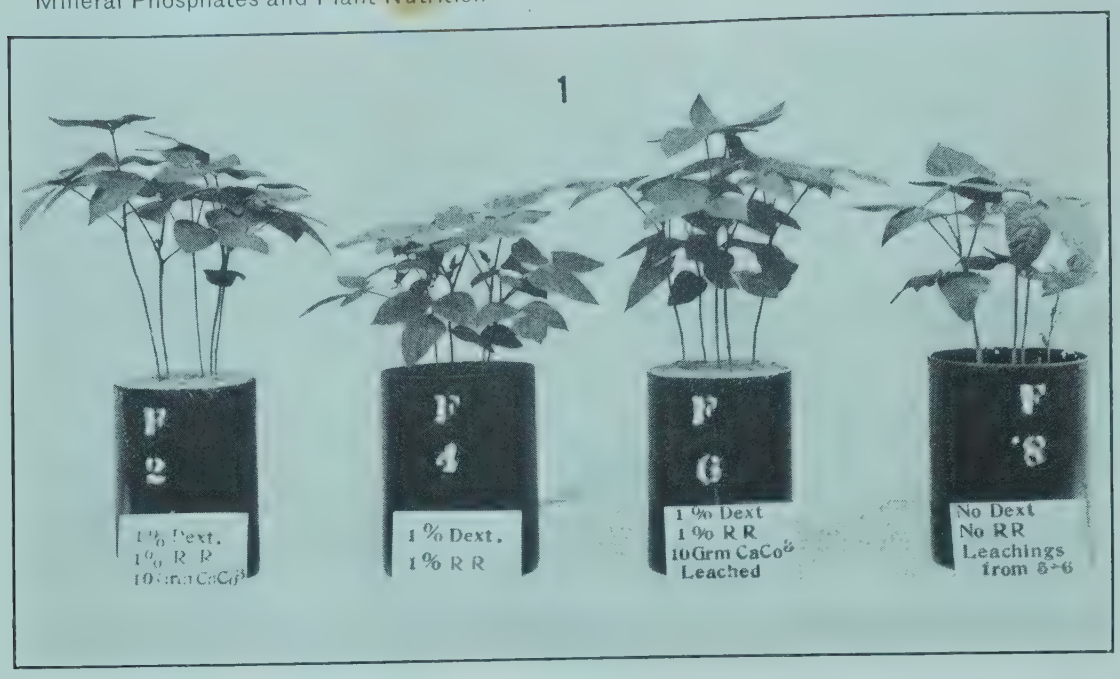
## PLATE LXXVI

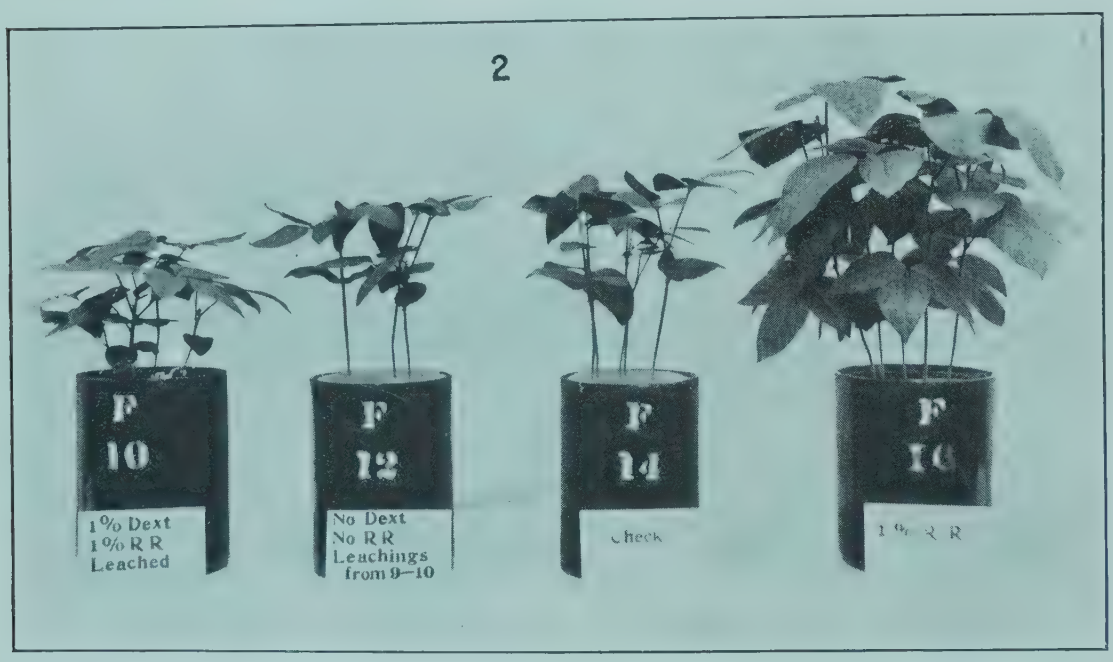
Effect of different kinds of mineral phosphate applied in different quantities for red clover. (Table VII.) Photograped just before first cutting.

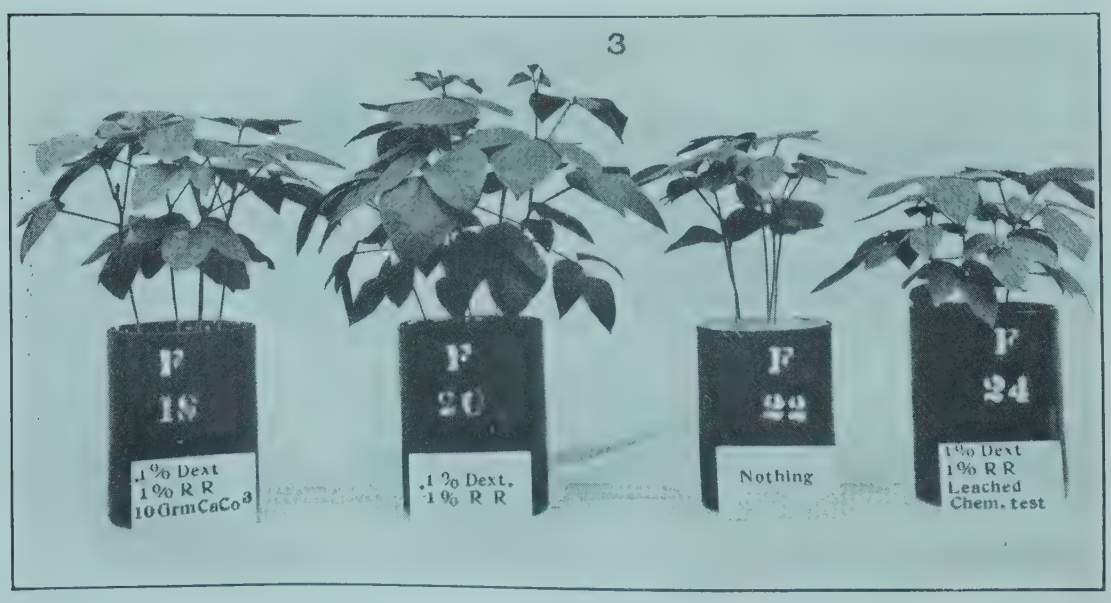
## PLATE LXXVII

Cowpeas, showing the comparative effect of Tennessee brown rock phosphate alone and in combination with dextrose. (Table XII.)

B
14
Check
B
11
1 % R R
B
1
.1 % Dext
1 % R R
B
4
.3 % Dext
1 % R R


1
F 2
1% Dext.
1% R R
F 4
1% Dext.
1% R R
F 6
1% Dext
1% R R
10 Grm CaCo3
Leached
F 8
No Dext
No RR
Leachings
from 5-6


2
F 10
1% Dext
1% R R
Leached
F 12
No Dext
No RR
Leachings
from 9–10
F 14
Check
F 16
1% R R


3
F 18
.1% Dext
1% R R
10 Grm CaCo3
F 20
.1% Dext.
1% R R
F 22
Nothing
F 24
1% Dext
1% R R
Leached
Chem. test

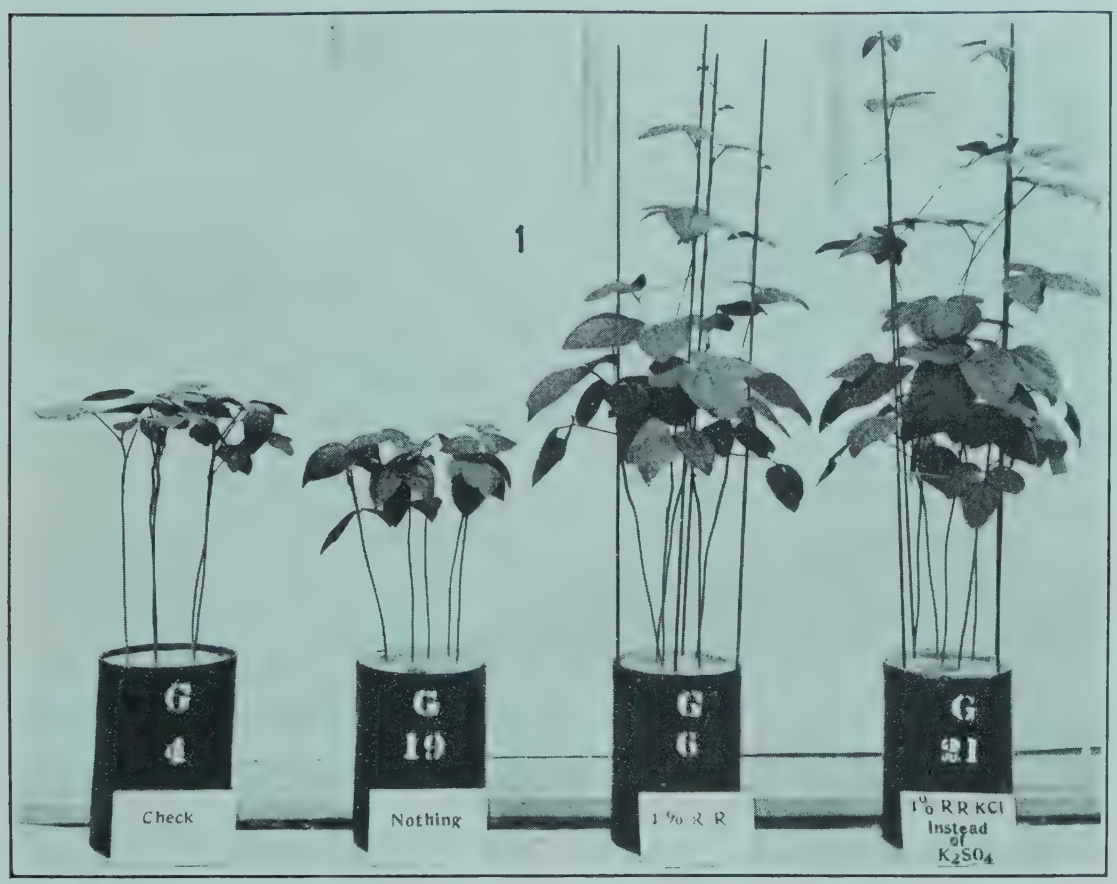
## PLATE LXXVIII

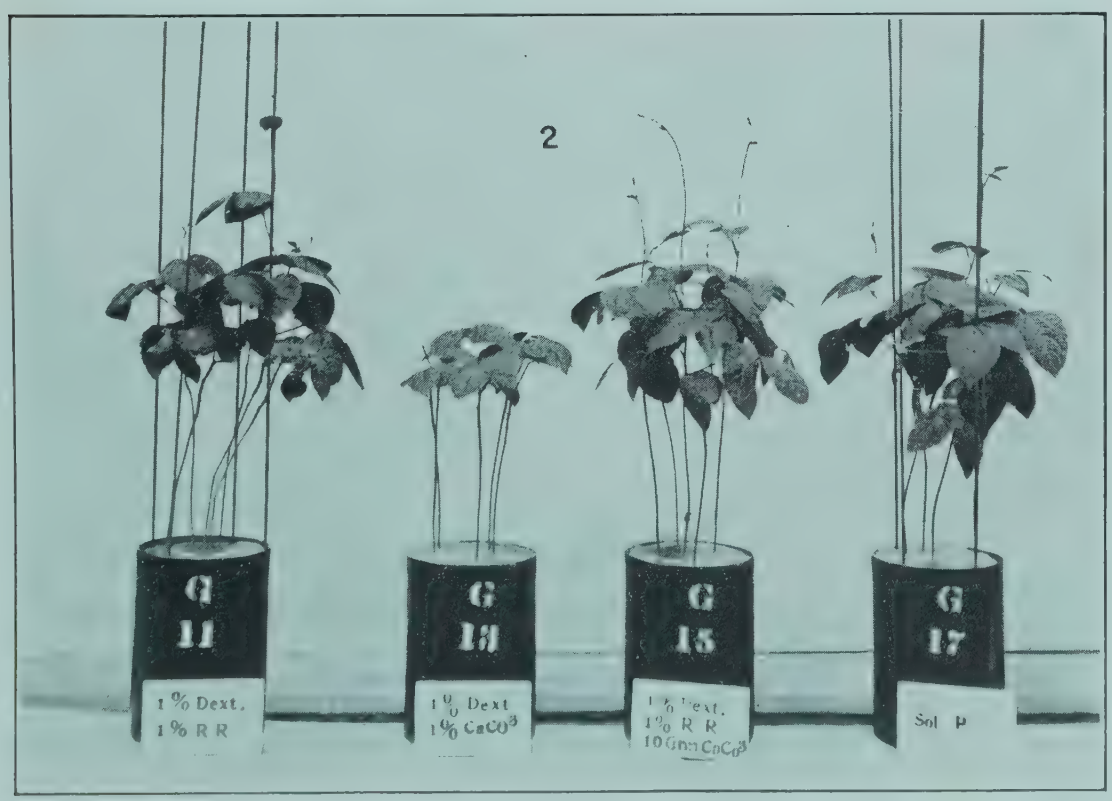
Cowpeas, showing the comparison of their growth when treated with Tennessee brown rock phosphate, phosphate and dextrose, and phosphate, dextrose, and calcium carbonate. (Table XIV.) Photographed just before harvesting.

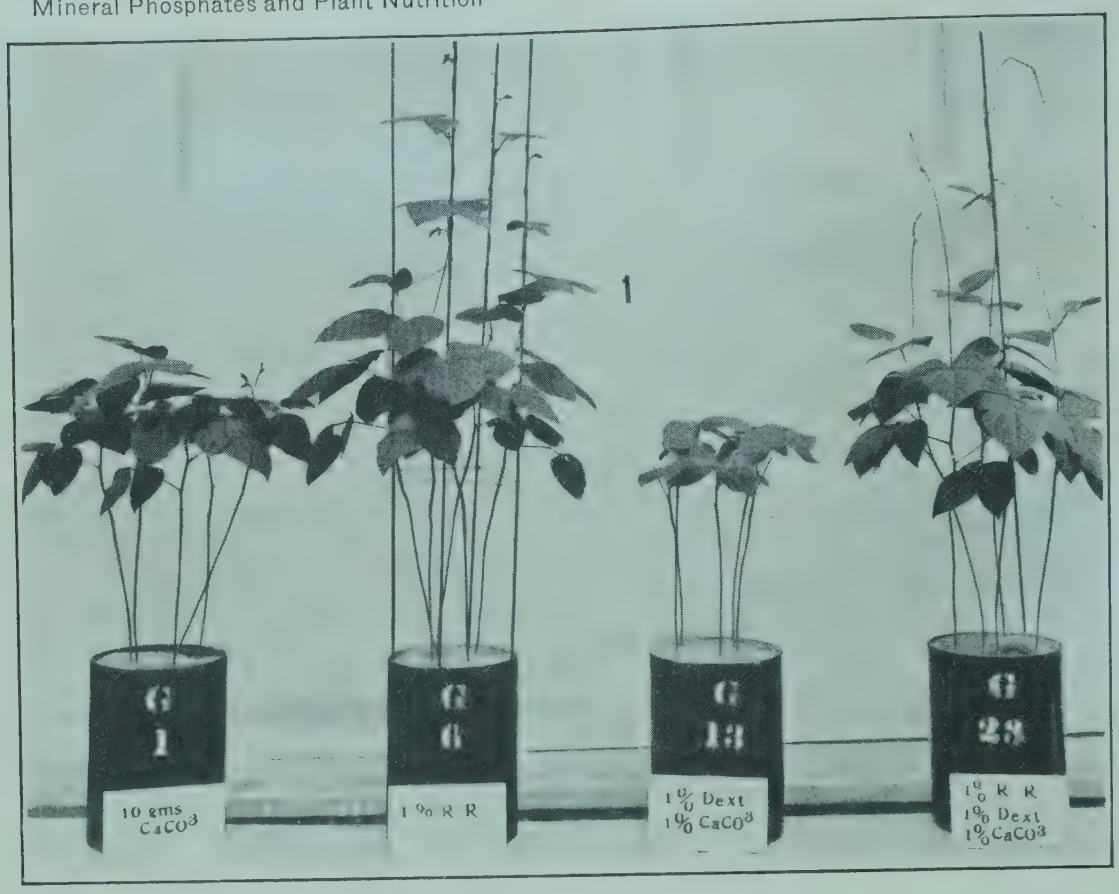
## PLATE LXXIX

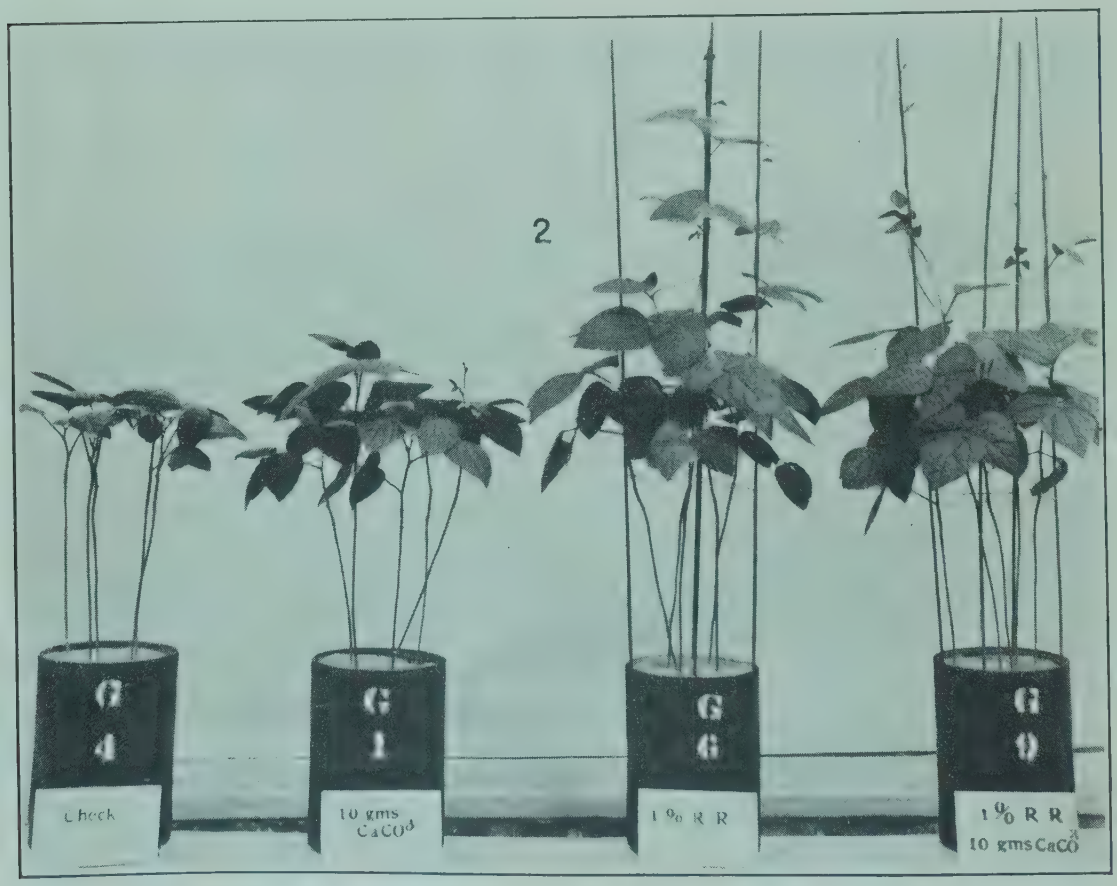
Effect of different substances on the growth of cowpeas:

Fig. 1.—Growth after the addition of varying quantities of raw rock. (Table XV.)
Fig. 2.—Growth after the addition of dextrose and soluble phosphate. (Table XV.)


1
G 4
Check
G 19
Nothing
G 6
1% R R
G 21
1% R R KCl Instead of $K_2SO_4$


2
G 11
1% Dext. 1% R R
G 13
1% Dext 1% $CaCO^3$
G 15
1% Dext. 1% R R 10 Grm $CaCO^3$
G 17
Sol P


1
G 1
10 gms $CaCO^3$
G 6
1% R R
G 13
1% Dext
1% $CaCO^3$
G 28
1% R R
1% Dext
1% $CaCO^3$


2
G 4
Check
G 1
10 gms $CaCO^3$
G 6
1% R R
G 9
1% R R
10 gms $CaCO^3$

## PLATE LXXX

Effect of various substances and combinations on the growth of cowpeas:

Fig. 1.—Effect of adding lime, phosphate rock, dextrose and lime, and phosphate rock, dextrose, and lime to the soil. (Table XV.)

Fig. 2.—Effect of adding nothing, lime, phosphate rock, and phosphate rock and lime to the soil. (Table XV.)

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SEP 6 1916

# CALIFORNIA GREEN LACEWING FLY

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## INTRODUCTION

The green lacewing fly (*Chrysopa californica* Coquillett) (fig. 1) has been observed by the writer on many occasions during the past five years in connection with outbreaks of aphids in southern Arizona and California, and at various times the extreme usefulness of the species in controlling these outbreaks has been noted. An opportunity for making

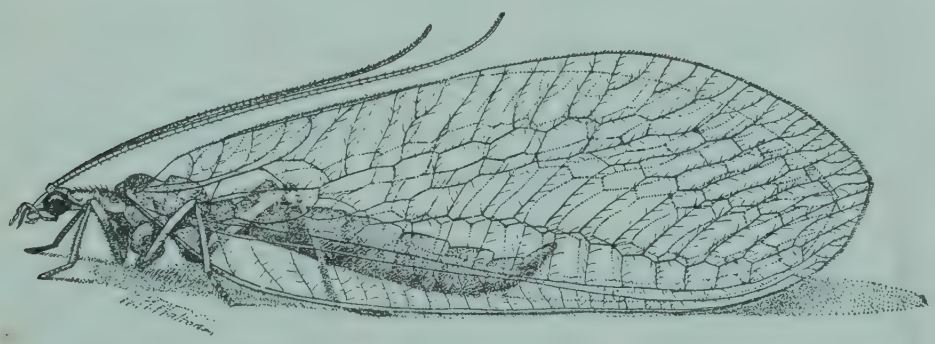

FIG. 1.—The California green lacewing fly (*Chrysopa californica*): Adult.

a complete study of the species came to hand during the past year (1915), and this paper is prepared for the purpose of recording the facts as observed and interpreted during this study.

## HISTORICAL REVIEW

This lacewing fly has been known since 1890, when it was collected in California and described by Coquillett (3)[1] in the Report of the State Board of Horticulture of that State. The next reference (1, p. 156) to it is in Banks's Revision of the Nearctic Chrysopidae. In this paper, published in 1903, Mr. Banks redescribed the species and stated that "it is the most abundant species on the Pacific coast."

It was not, however, until the year 1912 that the real usefulness and economic value of this species was brought forth. In this year

[1] Reference is made by number to "Literature cited," p. 524.

Quayle (7) listed it as an enemy of the Citrus red spider (*Tetranychus mytilaspidis* Riley) and remarked that "this is the commonest of the predatory insects occurring on citrus trees." Two years later the species was mentioned as an enemy of the Citrus red spider by Ewing (6). Essig (4), in 1911, described it briefly and included a few remarks on its habits and hosts. In 1913 (5), in his "California Insects," he credited this species of Chrysopa with feeding upon 14 different species of insects.

In August (2), 1915, and again in October (2), Mr. E. J. Branigan, a deputy of the California Horticultural Commission, referred to the economic importance of this species. In the first citation he reported the insect as feeding upon the "elm-leaf cluster louse." He stated: "Large numbers of the egg clusters of *Chrysopa californica* were present, the larvæ upon hatching, burrowing into the leaf clusters and feeding upon the lice." In October he reported this lacewing larva as attacking a citrus mealy bug (*Pseudococcus* sp.), and states further: "The green lacewing was found to be heavily parasitized by several species of parasites."

## DISTRIBUTION OF THE FLY

From our present knowledge of the species it is distinctly of western distribution, occurring throughout the Pacific Coast States, Texas, Arizona, New Mexico, Nevada, Lower California, and doubtless Utah. As early as 1903, Banks (1) stated:

> I have seen specimens from many places; Los Angeles, Tehama, Wanona [Wawona?], San Bernardino, Palo Alto, San Mateo County, Santa Clara County and Siskiou [Siskiyou ?] County, mostly in July and August, but some in April; also from Hood River, Oregon, September; Pullman, Wash., July and August; and King's Canon, Ormsby County, Nevada, July.

Mr. C. N. Ainslie, of the Bureau of Entomology, has taken a specimen of *Chrysopa* sp. at Salt Lake City, Utah, which is without much doubt this species. The writer has taken or seen specimens in southern California, Lower California, Mexico, Arizona, New Mexico, and in many different localities in these States at elevations varying from sea level to 7,000 feet

## HOST INSECTS

While the larvæ of this lacewing fly, as well as Chrysopidae in general, feed primarily upon aphids, their good work is far from being restricted to this group of insects. Mites, leafhoppers, thrips, and doubtless many other insects sufficiently small to be easily captured and devoured are likewise eaten.

Essig (5) has shown the following 14 species of insects to be attacked by *Chrysopa californica:*

Clover mite (*Bryobia pratensis* Garman).
Two-spotted mite (*Tetranychus mytilaspidis* Riley).
Red spider (*T. telarius* Linnaeus).
Apple leafhopper (*Empoasca mali* Le Baron).

Grape leafhopper (*Typhlocyba comes* Say).
The pear Psylla (*Psylla pyricola* Foerster).
Mealy plum plant louse (*Hyalopterus arundinis* Fabricius).
Melon aphis (*Aphis gossypii* Glover).
Black peach aphis (*Aphis persicæ-niger* Erwin Smith).
Green Citrus plant louse (*Macrosiphum citrifolii* Ashmead).
Citrus mealy bug (*Pseudococcus citri* Risso).
Frosted scale (*Eulecanium pruinosum* Coquillett).
Red scale (*Chrysomphalus aurantii* Maskell).
Purple scale (*Lepidosaphes beckii* Newman).

Mr. E. G. Smyth, working at Tempe, Ariz., found larvæ of *C. californica* feeding also on the wheat thrips (*Euthrips tritici* Fitch), which they apparently preferred to the pea aphis (*Macrosiphum pisi* Kaltenbach). Mr. R. N. Wilson, also at the Tempe laboratory, observed larvæ of *C. californica* feeding upon the barley mite (*Notophallus viridis* Banks) and on the "green bug" (*Toxoptera graminum* Rondani), while the writer reared the species exclusively on the corn leaf aphis (*Aphis maidis* Fitch), it being a very important check upon this pest.

## LIFE HISTORY AND HABITS OF THE LACEWING FLY

### THE ADULT

As before stated, this species of Chrysopa was first described by Coquillett (3) in 1890, and later (in 1903) redescribed by Banks (1). The original description of the adult by Coquillett is as follows:

> Pale green, a yellowish white dorsal stripe extends from front of thorax to tip of abdomen; front of head whitish; an irregular wine-red stripe extends from each eye to the mouth, and on its hind border, next the eye, is a black streak; front corners of thorax marked with black. Antennæ pale yellowish, minutely ringed with white. Wings greenish hyaline, obtusely pointed at their tips; veins and veinlets wholly green; seven or eight of the veinlets along the hind edge of front wings before the tips are forked; stigma somewhat opaque, yellowish green; legs green, tarsi whitish, the tips brown. Eyes greenish golden, becoming glaucous brown after death. In dried specimens the green coloring becomes more yellowish and the tarsi assumes a slightly darker color than the tibiae. Length 9 to 10 mm. (about ⅜ of an inch); expands from 24 to 28 mm. (about one inch or slightly over).

The adults are delicate green, flitting creatures which dart up from the shady protecting vegetation as one walks along a fence row or through an alfalfa field. The males are slightly smaller than the females and appear more vivid in color. During the breeding season both are short-lived. Neither sex has ever been noted by the writer to feed in the adult stage, even when food was offered, and doubtless all of the lacewing flies take little or no food in this period of their existence.

Copulation takes place almost immediately after the adults have issued and become dry, and in all cases under observation the male was dead on the following day. Oviposition usually begins the day following copulation and may continue for a period of three or four days, or the full complement of eggs may be deposited in a single day. Four

females under observation (see Table I) laid an average of 30¾ eggs each, the record being 34, 25, 38, and 26 eggs, respectively. The females, after performing what is apparently their sole purpose in life, die within 24 to 36 hours after oviposition is completed. The adults are especially numerous in southern Arizona during February, March, April, and May, and again during October and November.

## THE EGG

The egg (fig. 2) is placed on a long stalk or pedicel, which is hair-like and about half an inch in length. The egg itself is oblong and very small; at first it is whitish, but in a day or two it darkens and thereafter until it hatches the segmentation of the developing larva is revealed through the eggshell. It has a button or lid at the upper end, which is slightly flattened, while the lower end tapers until it is barely larger than the stalk to which it is attached. The original description by Coquillett (3) is as follows:

FIG. 2.—*Chrysopa californica:* Eggs.

> Very pale blue, elongate-ovate, pointed at the base, the apex flattened and in its center is a white button-shaped object; surface minutely granulated; length, three and one-half hundredths of an inch; mounted on a bristle-like pedicle from thirteen to eighteen hundredths of an inch long.

The egg stage (see Table I) was found to vary, being from 6 to 12 days in duration under the temperatures at which the experiment was carried on. The average time required for the 122 eggs under observation was 8 days.

TABLE I.—*The egg stage of Chrysopa californica at Tempe, Ariz., in 1915*

| Female No. | Cage No. | Eggs. | | | | Length of stage. | Average mean temperature. |
|---|---|---|---|---|---|---|---|
| | | Date laid. | Number. | Date hatched. | Number. | | |
| | | | | | | *Days.* | *° F.* |
| 1 | T 78 | Feb. 11 | 11 | Feb. 23 | 11 | 12 | 53 |
| | T 79 | Feb. 12 | 7 | ..do | 1 | 11 | 53 |
| | | | | Feb. 24 | 6 | 12 | 53 |
| | T 80 | Feb. 13 | 15 | ..do | 10 | 11 | 53 |
| | | | | Feb. 25 | 5 | 12 | 53 |
| | T 81 | Feb. 14 | 1 | ...do | 1 | 11 | 53 |
| 2 | T 1159 | Oct. 12 | 19 | Oct. 19 | 19 | 7 | 66.5 |
| | T 1160 | Oct. 13 | 6 | Oct. 20 | 6 | 7 | 67 |
| 3 | T 1185 | ...do | 20 | ...do | 20 | 7 | 67 |
| | T 1186 | Oct. 14 | 13 | ...do | 13 | 6 | 68 |
| | T 1187 | Oct. 15 | 4 | Oct. 21 | 4 | 6 | 69 |
| | T 1189 | Oct. 16 | 1 | Oct. 23 | 1 | 7 | 70 |
| 4 | T 1190 | ...do | 15 | Oct. 22 | 14 | 6 | 70 |
| | T 1196 | Oct. 17 | 11 | Oct. 23 | 11 | 6 | 70.5 |
| Total or average | | | 123 | | 122 | 8 | |

### THE LARVA

The larva when first hatched (fig. 3) is a delicate, white, nearly colorless object, quite conspicuously hairy and with mandibles which are large in comparison to the size of the body, these being about one-fourth its entire length. Coquillett's (3) description follows:

> Mixed with a yellowish white and pinkish brown, the latter color forming a dorsal line and a series of lateral spots; along each side of the body is a row of yellowish white tubercles; head yellowish white, marked with two diverging black stripes on the top, and with a dusky streak each side, having in its middle a black dot; length, 7 mm. (A little over one-fourth of an inch.)

### LARVAL HABITS

The hatching process requires but a few minutes, but the larva rests on the empty eggshell for some time after emergence. When the eggshell becomes dry and hardened, the larva hastily crawls down the supporting egg stalk and eagerly begins searching for food. If small aphids or thrips nymphs are present, it quickly seizes one of these and begins feeding. If only full-grown and large aphids are present, it is more cautious, running in a circle around the tempting and monstrous meal or following the aphid, ever and anon stopping as if to consider whether or not it could safely attack a creature so many times larger than itself. Finally, however, its increasing hunger apparently overcomes all fear and it pounces on its prey. The aphid is lifted bodily off its feet, the lacewing larva all the time crushing, piercing, and sucking its prey. The larvæ of all lacewing flies extract their food from the host by piercing it with their long, powerful mandibles, which are hollow, the internal fluids of the host being rapidly absorbed through them. With abundant food present the larva grows rapidly and quickly takes on a robust appearance.

FIG. 3.—*Chrysopa californica:* First instar.

### LARVAL DEVELOPMENT

The larvæ in the course of their development molt twice, which divides the larval period into three instars, with a total length of from 11 to 22 days, depending upon the prevailing temperature, the average length being about 16 days. (See Table II.) During this period from 74 to 160 full-grown aphids were eaten by each larva, the number consumed depending upon the temperature, the larvæ being more active and voracious during warmer weather.

TABLE II.—*Table of molts and instars of Chrysopa californica at Tempe, Ariz.*

PART I. FEBRUARY, 1915. AVERAGE MEAN TEMPERATURE, 54° F.

| Gage No. | Date hatched. | Date of first molt. | Length of first instar. | Number of aphids eaten. | Date of second molt. | Length of second instar. | Number of aphids eaten. | Date in cocoon. | Length of third instar. | Number of aphids eaten. | Total number of aphids eaten. | Total length of larval period. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Days.* | | | *Days.* | | | *Days.* | | | *Days.* |
| 78-1 | Feb. 23 | Mar. 3 | 8 | 15 | Mar. 10 | 7 | 10 | Mar. 17 | 7 | 43 | 68 | 22 |
| 78-2 | ...do.... | Mar. 2 | 7 | 15 | Mar. 9 | 7 | 15 | ...do.... | 8 | 59 | 89 | 22 |
| 78-3 | ...do.... | Mar. 3 | 8 | 17 | ...do.... | 6 | 12 | ...do.... | 8 | 61 | 90 | 22 |
| 78-5 | ...do.... | ...do.... | 8 | 17 | Mar. 11 | 8 | 16 | ...do.... | 6 | 50 | 83 | 22 |
| 79-1 | Feb. 24 | Mar. 4 | 8 | 12 | ...do.... | 7 | 16 | ...do.... | 6 | 47 | 75 | 21 |
| 79-2 | ...do.... | ...do.... | 8 | 10 | ...do.... | 7 | 16 | ...do.... | 6 | 51 | 77 | 21 |
| 79-5 | ...do.... | Mar. 5 | 9 | 12 | Mar. 12 | 7 | 23 | ...do.... | 5 | 39 | 74 | 21 |
| 80-3 | Feb. 25 | ...do.... | 8 | 12 | ...do.... | 7 | 22 | ...do.... | 5 | 49 | 83 | 20 |
| 80-5 | ...do.... | ...do.... | 8 | 11 | ...do.... | 7 | 19 | Mar. 18 | 6 | 74 | 104 | 21 |
| 80-6 | ...do.... | ...do.... | 8 | 10 | ...do.... | 7 | 15 | Mar. 17 | 5 | 56 | 81 | 20 |
| 80-7 | ...do.... | ...do.... | 8 | 13 | Mar. 11 | 6 | 25 | ...do.... | 6 | 62 | 100 | 20 |
| 80-9 | ...do.... | ...do.... | 8 | 14 | Mar. 12 | 7 | 27 | ...do.... | 5 | 56 | 97 | 20 |
| 80-10 | ...do.... | ...do.... | 8 | 14 | Mar. 11 | 6 | 28 | ...do.... | 6 | 63 | 105 | 20 |
| 80-11 | ...do.... | ...do.... | 8 | 13 | Mar. 12 | 7 | 20 | ...do.... | 5 | 56 | 89 | 20 |
| 80-12 | ...do.... | ...do.... | 8 | 15 | Mar. 15 | 10 | 32 | [1]Mar. 19 | ..... | 57 | 104 | ..... |
| Average | ..... | ..... | 8 | 13½ | ..... | 7 | 20 | ..... | 6 | 55½ | 88 | 20⅙ |

PART II. OCTOBER, 1915. AVERAGE MEAN TEMPERATURE, 70° F.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 59-2 | Oct. 19 | Oct. 23 | 4 | 14 | Oct. 26 | 3 | 21 | Oct. 30 | 4 | 108 | 143 | 11 |
| 59-3 | ...do.... | ...do.... | 4 | 17 | Oct. 27 | 4 | 33 | Nov. 1 | 5 | 70 | 120 | 13 |
| 59-4 | ...do.... | ...do.... | 4 | 16 | Oct. 26 | 3 | 18 | ...do.... | 6 | 109 | 143 | 13 |
| 59-6 | ...do.... | ...do.... | 4 | 15 | ...do.... | 3 | 34 | Oct. 31 | 5 | 100 | 147 | 12 |
| 59-7 | ...do.... | ...do.... | 4 | 13 | Oct. 27 | 4 | 35 | ...do.... | 4 | 95 | 143 | 12 |
| 59-9 | ...do.... | ...do.... | 4 | 13 | ...do.... | 4 | 35 | ...do.... | 4 | 90 | 139 | 12 |
| 59-10 | ...do.... | ...do.... | 4 | 15 | ...do.... | 4 | 26 | ...do.... | 4 | 93 | 134 | 12 |
| 59-11 | ...do.... | ...do.... | 4 | 16 | Oct. 26 | 3 | 30 | ...do.... | 5 | 114 | 160 | 12 |
| 59-12 | ...do.... | ...do.... | 4 | 14 | Oct. 27 | 4 | 36 | ...do.... | 4 | 91 | 141 | 12 |
| 59-13 | ...do.... | ...do.... | 4 | 14 | ...do.... | 4 | 32 | ...do.... | 4 | 100 | 146 | 12 |
| 59-14 | ...do.... | ...do.... | 4 | 12 | ...do.... | 4 | 39 | ...do.... | 4 | 107 | 158 | 12 |
| Average | ..... | ..... | 4 | 14⅓ | ..... | 3⅔ | 31 | ..... | 4½ | 98 | 143 | 12 |

[1] Died.

From these records it is seen that a *C. californica* larva under natural conditions, eating both large and small aphids, must often consume 300 or 400 of them during the course of its development. The economic value of these larvæ is thus seen to be enormous. It was found that an average of about 14 full-grown adults of *Aphis maidis* were consumed in the first instar, 4 to 7 aphids being eaten the first day after hatching. The duration of the first instar was found to vary with the temperature, it being from 4 to 9 days and the average period about 6 days in length. A great many more aphids are consumed during the second instar than in the first. This instar averages nearly a day shorter, being 7 days during March and 3⅔ days during October, 20 being the average number of aphids eaten by each of 15 larvæ during the former period and 31 during the latter. In actions and habits it is largely the same as the first instar except for the increased power of destroying aphids

The third-instar larvæ (fig. 4), while having a period of life averaging about the same in length as that of the second instar, make up for it in the number of aphids consumed. Fifteen larvæ in March each ate an average of 9½ aphids a day or 55½ during the entire period; whereas 11 larvæ each ate an average of nearly 22 full-grown aphids a day or 98 for the third-instar period, this being nearly twice as many as are eaten during the first and second instars. In Table IV it will be noted that a third-instar larva of *C. californica* in cage 59–14 ate 40 full-grown *Aphis maidis* in one day of 24 hours. The average length of the third instar was 6 days in March and 4½ days in October.

FIG. 4.—*Chrysopa californica:* Third instar.

Tables III and IV show the daily consumption of aphids by 26 larvæ during their entire larval period.

TABLE III.—*Daily feeding record of 15 larvæ of Chrysopa californica at Tempe, Ariz., in February, 1915*[a]

| Cage No. | Date hatched. | February. | | | | | March. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 | 25 | 26 | 27 | 28 | 1 | 2 | 3 | 4 | 5 | 6 |
| 78–1 | Feb. 23 | 5 | 5 | 2 | 2 | 0 | 0 | 1 | *m* 2 | 3 | 3 | 0 |
| 78–2 | do | 4 | 4 | 4 | 1 | 2 | 0 | *m* 0 | 5 | 5 | 5 | 0 |
| 78–3 | do | 5 | 3 | 5 | 2 | 0 | 0 | 2 | *m* 3 | 4 | 5 | 0 |
| 78–5 | do | 4 | 5 | 2 | 3 | 0 | 1 | 2 | *m* 0 | 3 | 5 | 2 |
| 79–1 | Feb. 24 | ..... | 2 | 4 | 2 | 2 | 0 | 0 | 2 | *m* 1 | 3 | 5 |
| 79–2 | do | ..... | 2 | 2 | 3 | 1 | 1 | 0 | 1 | *m* 0 | 4 | 8 |
| 79–5 | do | ..... | 4 | 3 | 0 | 3 | 2 | 0 | 0 | 0 | *m* 5 | 5 |
| 80–3 | Feb. 25 | ..... | ..... | 3 | 4 | 1 | 3 | 1 | 0 | 0 | *m* 3 | 4 |
| 80–5 | do | ..... | ..... | 3 | 1 | 4 | 2 | 0 | 0 | 1 | *m* 0 | 7 |
| 80–6 | do | ..... | ..... | 2 | 2 | 2 | 3 | 0 | 1 | 0 | *m* 4 | 3 |
| 80–7 | do | ..... | ..... | 3 | 2 | 3 | 4 | 0 | 1 | 0 | *m* 4 | 8 |
| 80–9 | do | ..... | ..... | 4 | 3 | 1 | 4 | 1 | 0 | 1 | *m* 2 | 7 |
| 80–10 | do | ..... | ..... | 4 | 3 | 1 | 4 | 2 | 0 | 0 | *m* 5 | 7 |
| 80–11 | do | ..... | ..... | 4 | 2 | 0 | 5 | 2 | 0 | 0 | *m* 4 | 6 |
| 80–12 | do | ..... | ..... | 4 | 5 | 0 | 3 | 3 | 0 | 0 | *m* 3 | 3 |

| Cage No. | Date hatched. | March. | | | | | | | | | | | | | | Average mean temperature. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | |
| | | | | | | | | | | | | | | | | °F. |
| 78–1 | Feb. 23 | 2 | 0 | 0 | *m* 3 | 7 | 7 | 8 | 7 | 7 | 4 | *c* | .... | .... | .... | 54 |
| 78–2 | do | 0 | 0 | *m* 5 | 10 | 10 | 10 | 10 | 8 | 4 | 2 | *c* | .... | .... | .... | 54 |
| 78–3 | do | 0 | 0 | *m* 3 | 9 | 10 | 10 | 10 | 10 | 4 | 5 | *c* | .... | .... | .... | 54 |
| 78–5 | do | 5 | 1 | 0 | 0 | *m* 5 | 10 | 9 | 8 | 8 | 10 | *c* | .... | .... | .... | 54 |
| 79–1 | Feb. 24 | 6 | 1 | 0 | 0 | *m* 3 | 9 | 9 | 9 | 8 | 9 | *c* | .... | .... | .... | 54 |
| 79–2 | do | 3 | 1 | 0 | 0 | *m* 8 | 9 | 10 | 10 | 10 | 4 | *c* | .... | .... | .... | 54 |
| 79–5 | do | 6 | 6 | 0 | 0 | 1 | *m* 4 | 10 | 10 | 8 | 7 | *c* | .... | .... | .... | 54 |
| 80–3 | Feb. 25 | 5 | 7 | 3 | 0 | 0 | *m* 3 | 10 | 12 | 10 | 14 | *c* | .... | .... | .... | 54 |
| 80–5 | do | 0 | 8 | 2 | 2 | 0 | *m* 6 | 10 | 13 | 15 | 15 | 15 | *c* | .... | .... | 54 |
| 80–6 | do | 5 | 3 | 0 | 0 | 0 | *m* 8 | 8 | 12 | 14 | 14 | *c* | .... | .... | .... | 54 |
| 80–7 | do | 7 | 6 | 0 | 0 | *m* 0 | 10 | 10 | 13 | 15 | 14 | *c* | .... | .... | .... | 54 |
| 80–9 | do | 6 | 8 | 3 | 1 | 0 | *m* 8 | 9 | 12 | 12 | 15 | *c* | .... | .... | .... | 54 |
| 80–10 | do | 8 | 7 | 0 | 1 | *m* 0 | 8 | 10 | 15 | 15 | 15 | *c* | .... | .... | .... | 54 |
| 80–11 | do | 2 | 7 | 1 | 0 | 0 | *m* 6 | 8 | 12 | 15 | 15 | *c* | .... | .... | .... | 54 |
| 80–12 | do | 7 | 5 | 0 | 4 | 8 | 2 | 0 | 0 | *m* 8 | 12 | 12 | 12 | 13 | *c* | 54 |

[a] *m*, Date of molting; *c*, date of spinning cocoon.

TABLE IV.—*Daily feeding record of 11 larvæ of Chrysopa californica at Tempe, Ariz., in October, 1915* [a]

| Cage No. | Date hatched. | October. | | | | | | | | | | | November. | Average mean temperature. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 1 | |
| | | | | | | | | | | | | | | | °F. |
| 59-2 | Oct. 19 | 7 | 7 | 0 | *m* 6 | 15 | 0 | *m* 18 | 20 | 25 | 25 | *c* 20 | .... | ..... | 70 |
| 59-3 | do | 7 | 7 | 3 | *m* 7 | 14 | 12 | 0 | *m* 16 | 23 | 28 | 3 | 0 | *c* | 70 |
| 59-4 | do | 5 | 7 | 4 | *m* 7 | 11 | 0 | *m* 17 | 19 | 22 | 26 | 25 | 0 | *c* | 70 |
| 59-6 | do | 7 | 7 | 1 | *m* 7 | 15 | 12 | *m* 10 | 18 | 25 | 25 | 22 | *c* | ..... | 70 |
| 59-7 | do | 6 | 7 | 0 | *m* 7 | 14 | 14 | 0 | *m* 20 | 24 | 23 | 28 | *c* | ..... | 70 |
| 59-9 | do | 6 | 5 | 2 | *m* 7 | 14 | 14 | 0 | *m* 10 | 25 | 25 | 30 | *c* | ..... | 70 |
| 59-10 | do | 7 | 6 | 2 | *m* 6 | 15 | 5 | 0 | *m* 19 | 24 | 26 | 24 | *c* | ..... | 70 |
| 59-11 | do | 7 | 7 | 2 | *m* 7 | 15 | 8 | *m* 14 | 20 | 25 | 25 | 30 | *c* | ..... | 70 |
| 59-12 | do | 7 | 7 | 0 | *m* 7 | 15 | 14 | 0 | *m* 20 | 25 | 23 | 23 | *c* | ..... | 70 |
| 59-13 | do | 5 | 7 | 2 | *m* 6 | 15 | 11 | 0 | *m* 20 | 25 | 25 | 30 | *c* | ..... | 70 |
| 59-14 | do | 6 | 6 | 0 | *m* 7 | 15 | 17 | 0 | *m* 19 | 23 | 25 | 40 | *c* | ..... | 70 |

[a] *m*, Date of molting; *c*, date of spinning cocoon.

Table III shows the record of 15 larvæ during the month of February and Table IV shows the record of 11 larvæ during the month of October. It will be noted that both the daily and total consumption were much larger during the latter and warmer period than during the former and that the total feeding period was nearly half the length during this period. Only full-grown wingless specimens of *Aphis maidis* were used in this experiment.

FIG. 5.—*Chrysopa californica:* Pupal case.

MOLTING

When the larva gets ready to molt, it settles down in some protected spot and rests for a period of several hours, often a day or more, and when the opportune time seems to have arrived it begins a series of movements, mostly of a rising and falling nature, calculated to burst the skin on the back. When this is finally accomplished, it crawls out and, after a few minutes' rest, is the same voracious creature it was before except only that its size is greater than in the preceding instar.

During the first and second instars, after the larva has eaten its quota of aphids, it rests, often as long as two days; during the last instar, however, this rest period is not apparent, owing to the fact that it takes place within the cocoon previous to pupation.

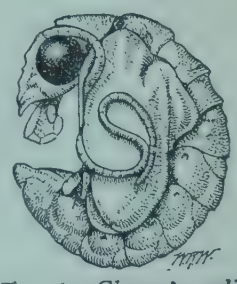

FIG. 6.—*Chrysopa californica:* Pupa.

As shown by dissections of several cocoons, this resting period, during which the pupa is forming within the larval skin, is from 6 to 9 days in length. Later in the observations it was discovered that one could tell by external indications just when this change

takes place. The larval skin when shed by the pupa is circular in form and is pressed firmly against one end of the pupal case, appearing from without and through the wall of the cocoon (fig. 5) as a dark, almost black, disk.

FIG. 7.—*Chrysopa californica:* Pupa freshly emerged from its cocoon.

### THE PUPA

The pupa (fig. 6) is formed within a membranous case or cocoon (fig. 5, 7) which is nearly globular in shape, tough but pliable, and inclosed or surrounded by numerous white filaments which hold it in place on the leaf or in some protecting cavity. The cases are often found singly, but when the infestation has been heavy, they may be in groups of a dozen or more. Mr. L. J. Hogg, an assistant, found as many as a dozen or more in a single curled ash leaf, the larvæ having fed on the elm-leaf cluster aphis.

TABLE V.—*Length of the pupal stage of Chrysopa californica at Tempe, Ariz., in 1915*

| Cage No. | Date of pupation. | Date adult issued. | Stage length. | Average mean temperature. |
|---|---|---|---|---|
| | | | *Days.* | °F. |
| 78–2 | Mar. 17 | Apr. 1 | 15 | 63 |
| 78–3 | ...do | ...do | 15 | 63 |
| 78–5 | ...do | Apr. 2 | 16 | 63 |
| 79–1 | ...do | Apr. 4 | 18 | 63 |
| 79–5 | ...do | Apr. 7 | 21 | 63 |
| 80–3 | ...do | Apr. 2 | 16 | 63 |
| 80–5 | Mar. 18 | Apr. 5 | 18 | 63 |
| 80–6 | Mar. 17 | Apr. 1 | 15 | 63 |
| 80–7 | ...do | ...do | 15 | 63 |
| 80–10 | ...do | Mar. 31 | 14 | 63 |
| 80–11 | ...do | Apr. 3 | 17 | 63 |
| 59–2 | Oct. 30 | Nov. 19 | 19 | 57 |
| 59–3 | Nov. 1 | Nov. 20 | 20 | 57 |
| 59–4 | ...do | Nov. 21 | 21 | 57 |
| 59–6 | Oct. 31 | Nov. 20 | 20 | 57 |
| 59–7 | ...do | ...do | 20 | 57 |
| 59–9 | ...do | Nov. 21 | 21 | 57 |
| 59–10 | ...do | Nov. 19 | 19 | 57 |
| 59–11 | ...do | Nov. 22 | 22 | 57 |
| 59–12 | ...do | Nov. 19 | 19 | 57 |
| 59–13 | ...do | Nov. 22 | 22 | 57 |
| 59–14 | ...do | Nov. 20 | 20 | 57 |
| Average | | | 18 | |

The pupal stage in southern Arizona (see Table V) varied from 14 days to 23 days in length, the average being 16⅛ days for March and $20\frac{7}{11}$ days for November.

As has been mentioned, the larva, after constructing the pupal case, which often requires a day's time, may remain several days before pupating. The pupa when formed is curled up as shown in figure 6, with the abdomen closely folded between the large thick wing pads. When ready to change to an adult, the pupa emerges from the cocoon (see fig. 7) through a circular lid, and in from ½ hour to 2 hours the pupal skin is shed and the adult (fig. 1) comes forth. After a few minutes have been allowed for the expansion and drying of the wings, the lacewing fly is ready for flight.

## SEASONAL HISTORY AND HIBERNATION

From the writer's observations during the past year (1915) in the Salt River Valley of Arizona, there are at least six generations annually. The first covers the period from about February 15 to March 15, and the remaining generations follow one another every 40 to 45 days from then until late October, either the pupa or adults of the last generation going into hibernation at that time. Adults can be taken throughout the winter months, but eggs have never been secured until the advent of milder weather. Pupæ are often taken during any of the winter months in the Salt River Valley of Arizona, which has a mild winter climate.

## NATURAL ENEMIES OF THE LACEWING FLY

It seems that in California (2) the species is commonly attacked by several species of parasites, but no record of any parasite has been obtained during the present study, although abundant material of this lacewing fly was examined. Robber flies have been noted to catch the adults, and certain Hemiptera prey upon the larvæ, but with these exceptions this lacewing fly seems to be quite free and unmolested.[1]

According to the records of the Biological Survey, United States Department of Agriculture, the Western wood pewee (*Contopus richardsonii*) feeds upon the species at Pasadena, Cal.; and at East Bernard, Tex., the nighthawk (*Chordeiles virginianus*) was found feeding upon the species, the stomachs of two birds containing three and six adults, respectively.

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[1] Possibly this is due to the extremely offensive odor thrown off by the adults of all lacewing flies when alarmed.

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# RAPE AS MATERIAL FOR SILAGE

By A. R. Lamb, *Assistant Chemist*, and John M. Evvard, *Assistant Chief in Animal Husbandry, Iowa Agricultural Experiment Station*

## INTRODUCTION

The popularity of rape (*Brassica napus*) as a pasture crop has been steadily increasing since its introduction into this country about 25 years ago. Its value as such is considerable, but its usefulness would be greatly increased if it could be preserved in the silo and used successfully as a succulent feed for the winter months. Attempts to ensile it have, however, evidently been few, perhaps since it has generally been considered too watery for this purpose. The only report of such an attempt which has been found in the literature is from Canada.[1]

In that experiment rape was cut when about 15 inches high and ensiled alone and with an equal weight of corn. When the silage was fed six months later, it was said to have been well preserved, to have had a pleasant odor, and to have been eaten with avidity by cattle. Chemical analyses showed a considerable loss of water and carbohydrates and an increase in nonprotein nitrogen. With the exception of the loss of water, these losses are not much greater than the losses which occur in ensiling the corn plant (*Zea mays*). In that experiment the total loss of dry matter was 26.5 per cent. Weight for weight, however, rape silage was found by analysis to be a much more valuable feeding material than green rape.

In 1914, Evvard, at the Iowa Station,[2] made rape silage in barrels, with and without the addition of common salt (sodium chlorid). The highly salted silage was quite well preserved and had a favorable odor, but was refused by stock. The unsalted silage contained mold and had undergone some putrefactive fermentation, the odor of volatile sulphids being quite evident. The shape of the barrels and the consequent difficulty of excluding air on the settling of the ensiled material were responsible for this putrefaction. This emphasizes the importance of using suitable air-tight containers in making rape silage.

The ideal plant for silage making must contain just sufficient fermentable sugars to furnish enough acids to preserve it. In most respects the corn plant furnishes the most nearly ideal material for silage. The legumes are not ensiled so successfully because the percentage of protein is too high for the amount of sugar, and some putrefaction is likely to

[1] Schutt, F. T. Report of the chemist. Fodders and feeding stuffs. *In* Canada Exp. Farms Rpts., 1904, p. 166–182. 1905.

[2] Unpublished data.

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occur. Rape contains a larger amount of sugars[1] and is therefore likely to develop a high acidity. Rape, in common with other Cruciferae, contains considerable amounts of organic sulphur compounds, which are likely to form disagreeable volatile products if the fermentation progresses too far. For these reasons a mixture of rape and a legume should produce better silage than either alone.

## EXPERIMENTAL RAPE SILAGE

The experimental silage was therefore made from rape alone and from mixtures of rape with various other materials, as outlined in Table I, with the purpose of determining the most satisfactory combination. The other plant materials used were alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), sweet clover (*Melilotus alba*), potato tubers (*Solanum tuberosum*), timothy (*Phleum pratense*), Sudan grass (*Andropogon sorghum, aethiopicus*), sorghum cane (*Sorghum vulgare*), and bluegrass (*Poa pratensis*). The rape used was quite mature but still succulent. The rape leaves were cut off at the main stalk. The entire plant was cut 3 inches from the ground. The alfalfa was cut just before blooming. The corn, Sudan grass, and sorghum cane used were mature. The other plant materials were cut just before maturity. All the forage was cut by a silage cutter into half-inch lengths. The material was tightly packed into glass jars of about 1-gallon capacity, in the same manner as corn silage has repeatedly been made in this laboratory. The jars were closed with metal caps, which were not too tight to prevent the escape of excess gases.

[1] An average air-dry sample contains 5.60 per cent of total fermentable sugars calculated as dextrose.

TABLE I.—*Analyses of rape silage*

| Sample No. | Percentage of constituents of sample. | Result of inspection. | | | | Water. | Data on 100 c. c. of juice. | | | | | | | Calculated to 100 gm. of dry matter. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total acidity. | | Volatile acidity. | | Fixed acidity (by difference) calculated as lactic acid. | Alcohol. | Amino nitrogen. | Total acidity calculated as lactic acid. | Volatile acidity calculated as acetic acid. | Fixed acidity (by difference) calculated as lactic acid. | Alcohol. | Amino nitrogen. |
| | | Appearance. | Odor. | Taste. | Class. | | N/10 solution. | Calculated as lactic acid. | N/10 solution. | Calculated as acetic acid. | | | | | | | | |
| | | | | | | *Per ct.* | *C. c.* | *Gm.* | *C. c.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | Rape entire | Good | Pleasant | Sour | B | 84.5 | 275 | 2.475 | | | | | | 13.572 | | | | |
| 2 | Rape leaves | do | do | Very sour | C | 86.0 | 298 | 2.682 | 76.8 | 0.461 | 1.989 | [a] 0.046 | 0.281 | 16.452 | 2.827 | 12.213 | 0.220 | 1.724 |
| 3 | Rape 80, alfalfa 20 | do | Aromatic | do | B | 84.7 | 252 | 2.268 | | | | | | 12.528 | | | | |
| 4 | Rape 60, alfalfa 40 | do | Unpleasant | Disagreeable [b] | E | 83.3 | 189 | 1.701 | | | | | | 8.505 | | | | |
| 5 | Rape 40, alfalfa 60 | do | Aromatic | Good, but sour | A | 83.1 | 227 | 2.043 | | | | | | 10.062 | | | | |
| 6 | Rape leaves 80, alfalfa 20 | do | Sauerkraut | Very sour | C | 85.8 | 250 | 2.250 | 128.2 | .769 | 1.093 | [a] .060 | .268 | 13.554 | 4.634 | 6.606 | .361 | 1.614 |
| 7 | Rape leaves 60, alfalfa 40 | do | do | Good, but too sour. | B | 85.5 | 232 | 2.088 | | | | | | 12.276 | | | | |
| 8 | Rape leaves 40, alfalfa 60 | do | Aromatic | Favorable | A | 82.2 | 269 | 2.421 | | | | | | 11.151 | | | | |
| 9 | Rape leaves 90, cane sugar 10 | do | do | Pleasant, but very sour. | C | 81.8 | 342 | 3.078 | 69.2 | .415 | 2.457 | [c] 3.986<br>[a] 3.960 | .280 | 13.860 | 1.870 | 11.052 | 17.954<br>17.838 | 1.252 |
| 10 | Rape leaves 90, molasses 10 | Dark color | Agreeable | Too sour | C | 84.3 | 301 | 2.709 | 48.8 | .293 | 2.268 | [c] 2.011 | | 14.562 | 1.574 | 12.204 | 10.812 | |
| 11 | Rape 90, molasses 10 | do | do | do | C | 82.1 | 351 | 3.159 | | | | | | 14.490 | | | | |
| 12 | Rape leaves 80, molasses 20 | Dark | Like molasses | Sour | C | 82.1 | 345 | 3.105 | | | | | | 14.238 | | | | |
| 13 | Rape 80, starch 20 | do | Starchy (slimy) | do | D | 75.4 | 234 | 2.106 | | | | | | 6.453 | | | | |
| 14 | Rape leaves 80, starch 20 | do | do | Less sour | D | 77.2 | 123 | 1.107 | | | | | | 3.744 | | | | |
| 15 | Rape leaves 60, potatoes 40 | Good | Sour | Not bad | C | 84.7 | 188 | 1.692 | | | | | | 9.342 | | | | |
| 16 | Rape leaves 80, corn grain 20 | do | Good, but sour | Very sour | C-D | 69.9 | 355 | 3.195 | | | | | | 7.407 | | | | |
| 17 | Rape leaves 60, whole corn plant 40 | do | Pleasant | do | C | 79.4 | 342 | 3.078 | | | | | | 11.880 | | | | |
| 18 | Rape 60, corn grain 40 | do | Agreeable | Good, but sour | B | 58.3 | 442 | 3.978 | 90.5 | .543 | 3.159 | [a] .424 | .460 | 5.562 | .760 | 4.419 | .593 | .643 |

[a] By aeration. [b] Possibly accidental contamination. [c] By distillation.

TABLE I.—*Analyses of rape silage*—Continued

| Sample No. | Percentage of constituents of sample. | Result of inspection. | | | | Water. | Data on 100 c. c. of juice. | | | | | | | Calculated to 100 gm. of dry matter. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total acidity. | | Volatile acidity. | | | | | | | | | |
| | | Appearance. | Odor. | Taste. | Class. | | *N*/10 solution. | Calculated as lactic acid. | *N*/10 solution. | Calculated as acetic acid. | Fixed acidity (by difference) calculated as lactic acid. | Alcohol. | Amino nitrogen. | Total acidity calculated as lactic acid. | Volatile acidity calculated as acetic acid. | Fixed acidity (by difference) calculated as lactic acid. | Alcohol. | Amino nitrogen. |
| | | | | | | *Per ct.* | *C. c.* | *Gm.* | *C. c.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 19 | Rape leaves+100 gm. of calcium carbonate. | Dark | Disagreeable | "Rotten"[a] | F | ..... | 81 | 0.729 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 20 | Rape+water | .....do | Strong | Disagreeable | E | 90.7 | 131 | 1.179 | ..... | ..... | ..... | ..... | ..... | 11.439 | ..... | ..... | ..... | ..... |
| 21 | Rape leaves + water. | .....do | .....do | Disagreeable, sour. | E | 89.5 | 155 | 1.395 | ..... | ..... | ..... | ..... | ..... | 11.916 | ..... | ..... | ..... | ..... |
| 22 | Rape leaves 60, red clover 40. | Good | Aromatic and pleasant. | Very palatable | B | 83.2 | 272 | 2.448 | 73.6 | 0.442 | 1.782 | [b] 0.147 | 0.224 | 12.114 | 2.186 | 8.838 | 0.727 | 1.109 |
| 23 | Rape leaves 60, sweet clover 40. | .....do | Good (suggests sweet clover). | Bitter, sour | C | 84.1 | 272 | 2.448 | ..... | ..... | ..... | ..... | ..... | 12.951 | ..... | ..... | ..... | ..... |
| 24 | Rape leaves+lactic-acid culture. | Dark | Rather disagreeable. | Unpleasant, biting. | D | 86.3 | 222 | 1.998 | 70.9 | .425 | 1.359 | ..... | ..... | 12.564 | 2.675 | 8.550 | ..... | ..... |
| 25 | Rape leaves 60, blue grass 40. | Good | Good | Agreeable, but sour. | C | 77.3 | 321 | 2.889 | ..... | ..... | ..... | ..... | ..... | 9.819 | ..... | ..... | ..... | ..... |
| 26 | Rape leaves 60, timothy 40. | .....do | Aromatic | Sour | C | 79.9 | 341 | 3.069 | ..... | ..... | ..... | ..... | ..... | 14.472 | ..... | ..... | ..... | ..... |
| 27 | Rape 60, Sudan grass 40. | .....do | Aromatic like corn silage. | Slightly acid | B | 74.1 | 330 | 2.970 | ..... | ..... | ..... | ..... | ..... | 8.478 | ..... | ..... | ..... | ..... |
| 28 | Rape leaves 60, sorghum cane 40. | .....do | .....do | Sour | C | 74.4 | 329 | 2.961 | ..... | ..... | ..... | ..... | ..... | 8.604 | ..... | ..... | ..... | ..... |

[a] Crystals of calcium salts present.

[b] By aeration.

The jars were opened four months after filling and the condition, appearance, odor, and taste of the silage noted. (See Table I.) With very few exceptions it was in a perfect state of preservation, of excellent texture and color, with a pleasant, somewhat aromatic odor, and generally of an agreeable taste, though quite sour. It was succulent without being too moist, even though it had been made in a tightly sealed jar, with almost no opportunity for the evaporation of water. In order to ascertain its palatability to swine, a representative number of the various mixtures and some of the pure rape silage were fed to three lots of pigs. At first the animals, which were on a ration consisting mainly of corn and tankage, tasted the silage rather hesitatingly and seemed surprised by the sourness, but kept at it until they had eaten it all, appearing to enjoy its succulency. On a second trial, three days later, the same animals ate it with great relish. Only one sample of those tried, a rape-molasses mixture, was refused by the animals. In nearly every case after eating this silage they went to the corn self-feeders. An extensive feeding experiment to determine the effect of feeding rape silage upon the growth and well-being of swine is contemplated.

## CHEMICAL EXAMINATION OF SILAGE SAMPLES

The data from the chemical examination of the samples are shown in Table I. The juice was pressed out from the silage, and samples were taken from the juice with pipettes. This method has been used with corn silage and is a quite accurate and excellent comparative method for quickly determining the character of a sample of silage. Estimations of the total acidity and moisture content were made on all samples, and estimations of volatile acidity, alcohol, and amino nitrogen on a few representative samples, according to the following methods. (See Table I.)

TOTAL ACIDITY.—Ten c. c. of juice were diluted to about 500 c. c. with carbon-dioxid-($CO_2$)-free water, and titrated with decinormal barium-hydroxid solution in the presence of phenolphthalein till a distinct pink appeared by reflected light against a white background.

VOLATILE ACIDITY.—Fifty c. c. of juice were diluted to 100 c. c. with carbon-dioxid-free water and distilled with a current of carbon-dioxid-free steam. To hasten the liberation of volatile acids and alcohols, 100 gm. of sodium chlorid were added to the juice. About 500 c. c. of distillate were titrated with baryta water in the presence of phenolphthalein.

ALCOHOL.—Distillation method: The distillate from the volatile-acid determination was neutralized with baryta water (solid phenolphthalein being added) and concentrated by repeated distillation with sodium chlorid.[1]

About 50 c. c. of alcohol solution were oxidized [2] in a pressure flask in a boiling water bath for 30 to 40 minutes, and the volatile acids then distilled off four or five

[1] Bacon, R. F. Detection and determination of small quantities of ethyl and methyl alcohol and of formic acid. U. S. Dept. Agr. Bur. Chem. Circ. 74, 8 p. 1911.

[2] The oxidizing solution used was made up in the following proportions: 10 gm. $K_2Cr_2O_7$, 20 gm. $H_2SO_4$, 70 gm. water.

times, with additions of carbon-dioxid-free water. The total alcohols found were calculated as ethyl alcohol.

Aeration method: In this method a current of air was drawn through the alcohol solution, which was saturated with ammonium sulphate, into concentrated sulphuric acid. The sulphuric-acid solution was then oxidized with potassium-dichromate solution and distilled as before.

AMINO NITROGEN.—The amino nitrogen was determined on the diluted juice with the Van Slyke apparatus.[1]

MOISTURE.—The moisture content was determined by heating a sample of about 100 gm. in an oven at 100° C.

## DISCUSSION

The determinations of total acidity, volatile acidity, total alcohols, and amino nitrogen furnish a measure of the most characteristic changes which take place in silage fermentation and a partially complete picture of the character of the fermentation and the character of the silage, as nearly as chemical analysis can show. This, the ordinary estimations of crude protein, fiber, ether extract, and ash fail to do. The amount of amino nitrogen is, of course, of comparative value only, but it shows the degree of hydrolysis of protein. Unfortunately in this case no figures are now available for the amino nitrogen of green rape. The results given in Table I, however, indicate that the degree of hydrolysis of protein was nearly the same in each sample upon which this determination was made. The total acidity was quite similar in each of the samples which were classed "A" and "B." The total acidity of the silage juice in most cases is no higher than the average acidity of corn-silage juice. The average of analyses on 100 c. c. of juice of several samples of normal corn silage is as follows:

| | |
|---|---|
| Total acidity | 271 c. c. of *N*/10 solution. |
| Volatile acidity | 91 c. c. of *N*/10 solution. |
| Alcohol | 0.312 gm. |
| Amino nitrogen | 0.109 gm. |

The explanation of the very sour taste of rape silage may lie in the fact that it has a much higher water content than corn silage and thus affects the nerves of taste more quickly. A considerable amount of sulphates was found in one sample, but the presence of any free mineral acid could not be demonstrated. The volatile acidity seemed to vary more widely, with varying experimental conditions. The alcohol content was probably small in all cases where there was no addition of sugar. In two cases of silage with added sugar or molasses Table I shows that an abnormally large amount of alcohol was found. This, as well as the increased acidity, militates against the addition of molasses to silage materials. It is very probable that the excess alcohol was formed after the maximum acidity had been reached and the yeasts had gained the ascendancy.

[1] Van Slyke, D. D. The quantitative determination of aliphatic amino groups, II. *In* Jour. Biol. Chem., v. 12, no. 2, p. 275–284, 1 fig., 1 pl., 1 tab. 1912.

The classification of the samples as to general silage quality (A, B, C, etc.) is necessarily approximate. All the samples, however, could be classed as "good silage," except those rated below "D." Those containing fibrous material, such as sorghum cane, Sudan grass, timothy, and corn plant, would be useful for cattle, but would not be as good feed for swine as pure rape silage, or the alfalfa, red clover, potato, or corn-grain mixtures. The silage made from the entire rape plant was quite similar to that made from the leaves. However, for swine too much fiber is objectionable.

The mixtures of rape with legumes are perhaps best, from the standpoint of feeding as well as that of the quality of the silage. The rape improves the mixture, in that it supplies the necessary fermentable carbohydrates, which apparently are deficient in amount in the legume. In this connection it may be noted that since legume silage is not entirely satisfactory, it may be greatly improved by adding 20 per cent or more of rape, which would supply the necessary sugars. On general considerations the indications are that this sort of silage should be useful for either cattle, sheep, or swine. Practical farmers have sowed rape in the cornfield at the time of the last cultivation, it later being ensiled with the corn. This mixed silage has been fed to cattle with apparently good results.

## SUMMARY

(1) Rape was successfully ensiled in glass jars, alone and in mixtures with other materials.

(2) Excepting one or two mixtures, this silage was palatable to swine.

(3) Chemical examination of the samples showed the acidity and alcohol content to be comparable in most cases to that of corn silage.

(4) A mixture of rape and a legume produces the best quality of silage.

# EFFECT OF AUTOLYSIS UPON MUSCLE CREATIN

By RALPH HOAGLAND, *Senior Biochemist*, and C. N. MCBRYDE, *Senior Bacteriologist, Biochemic Division, Bureau of Animal Industry*[1]

## INTRODUCTION

The question as to the relation between muscle creatin and urinary creatinin is one which has been the subject of considerable investigation, particularly during the past few years. The importance of this problem lies in the fact that it is now quite clearly established that the creatinin excreted in the urine with a creatin-creatinin-free diet, is an accurate measure of endogenous metabolism. In the mammalian family, creatin is found chiefly in the striated muscular tissue, and to a lesser extent in other tissues and in fluids. The anhydrid creatinin is present in very small quantities. Since the amount of creatinin excreted in the urine is an accurate measure of tissue metabolism and since creatin is a normal constituent of muscular tissue, and since also there is a close chemical relationship between the two compounds, the natural supposition is that urinary creatinin is derived from muscle creatin. This may be said to be the generally accepted view, and it is supported by considerable experimental evidence; yet, on the other hand, certain investigators have obtained results which do not appear to support this theory.

The question as to where creatinin is formed in the body is another problem concerning which there is considerable lack of agreement. In the light of our present knowledge on the subject it must be admitted that the method and the place of production of creatinin in the body have not been clearly established.

In the course of a series of autolytic experiments with lean beef, carried on in connection with investigations concerning changes taking place in beef in cold storage, certain changes were noted in the creatin and creatinin content of the muscles which appear to throw some light on the question as to the source and method of production of creatinin. The results of these observations are offered as a contribution to our knowledge of the subject.

## PREVIOUS AUTOLYTIC EXPERIMENTS

Gottlieb and Stangassinger (3)[2] carried on an extensive series of autolytic experiments with various organs, tissues, and fluids of dogs, cats, and calves, using toluol as an antiseptic. As a result of their studies these

[1] The authors desire to extend their thanks to Mr. W. C. Powick for assistance rendered in connection with the analytical work reported in this paper.

[2] Reference is made by number to "Literature cited," p. 546.

authors came to the following conclusions: (1) Muscles and other tissues produce creatin in the early stages of autolysis; (2) natural and added creatin are changed in part to creatinin, owing to the action of dehydrating ferments; (3) creatin and creatinin are in part destroyed as autolysis progresses, owing to the action of ferments which they name "kreatase" and "kreatinase."

The work of these authors, so far as changes in free creatinin are concerned, is open to criticism on account of the method which they used for the determination of this constituent. The extracts were concentrated nearly to dryness on a steam bath, the solutions having been neutralized by the addition of barium carbonate. It is now recognized that such a method of concentrating a solution containing creatin will convert a part of that base into creatinin. For these reasons the work of Gottlieb and Stangassinger concerning the production of free creatinin during autolysis must be regarded as of doubtful value.

Stangassinger (9) studied the action of autolyzing body tissues and fluids upon added creatin and the effect of various chemicals and conditions upon the rate and extent of the reaction. Blood, kidneys, livers, and lungs of dogs were used in the experiments. The so-called dehydrating ferments kreatase and kreatinase were found to be most active in weak acid solutions, and toluol had the least retarding action of all the antiseptics used. Protoplasmic poisons checked the action of the ferments. Creatin was formed in the early stages of the autolysis of liver and blood, the material from well-fed animals containing larger amounts of creatin-forming material than that from hungry dogs. Liver extract destroyed added creatinin in appreciable quantities.

This author's findings concerning changes in free creatinin are open to the same criticisms as those made of the work of Gottlieb and Stangassinger (3).

Mellanby (4) carried on autolytic experiments with various tissues, but was unable to confirm in any respect Gottlieb and Stangassinger's findings (3) concerning the effect of autolysis of tissues upon creatin and creatinin. A careful examination of Mellanby's article indicates that his conclusions should not be taken too seriously. For example, rabbit muscle was autolyzed, under strictly aseptic conditions, for five days at 37° C., and at the end of that time no free creatinin could be detected. If it could not then be detected, it certainly could not be found in the author's other experiments, in which autolysis was carried on under less favorable conditions.

Rothmann (7) carried on a series of autolytic experiments in reply to Mellanby's criticism (4) of Gottlieb and Stangassinger's work (3). The work was conducted under strict bacteriological control and it was found that the liver, kidney, and blood of dogs destroyed appreciable quantities of creatin. He admits the correctness of Mellanby's criticism of Gottlieb and Stangassinger's method for the determination of free

creatinin, stating that in the operation creatin was probably changed in part to creatinin. However, using Mellanby's method for the determination of free creatinin, he found that liver and kidney extracts converted appreciable quantities of creatin into creatinin.

Pekelharing and Van Hoogenhuyze (6) found fairly marked increases in the creatin content of muscles on the completion of rigor mortis and heat rigor.

Rowe (8) carried on autolytic experiments with the parathyroids and adrenals of sheep and found that, in a marked degree, these tissues had the property of destroying added creatin. Thyroid extract destroyed 71 per cent of the added creatin in 48 hours and adrenal extract destroyed 69 per cent in 72 hours.

Myers and Fine (5) studied the effect of autolysis upon the creatin and creatinin content of various tissues and fluids of vertebrate animals. Very marked increases were noted in the creatinin content of all the materials examined after autolysis. Human blood and rabbit liver showed marked gains in the total creatinin. In the case of dog muscle an appreciable decrease in total creatinin was noted. The authors are of the opinion that muscular tissue is the site of creatinin formation.

## THE PRESENT EXPERIMENTS

Two series of autolytic experiments were carried on: One under aseptic conditions; the other with the use of antiseptics. It is generally recognized that the aseptic method is to be preferred, so far as the value of the results is concerned; but owing to the extreme care required in carrying on an autolytic experiment under aseptic conditions, the antiseptic method is commonly employed. In these investigations the antiseptic method was used simply as a check against the aseptic method, and for the purpose of comparison.

### ASEPTIC AUTOLYSIS EXPERIMENTS

A prime steer was slaughtered at a local abattoir by the usual methods under the personal supervision of one of the authors. It was, of course, impossible to carry out the operation of skinning under strictly aseptic conditions, so the chief aim was to make this operation as cleanly as possible. The entire carcass was first wet down to prevent the dissemination of dust particles. The carcass was kept suspended while it was being skinned and was not allowed to come into contact with the floor, which had also been washed to prevent dust from rising. In skinning the carcass, knives were used which had been dipped in boiling water, and they were again dipped from time to time in the boiling water. As soon as the skin was removed one of the hindquarters was wound with gauze which had been wrung out in a solution of mercuric chlorid (1:1,000); then it was separated from the body and completely enveloped in the

gauze. The hindquarter was next wrapped in dry cheesecloth and heavy paper and transported at once to the laboratory by motor truck, the trip requiring less than an hour.

### METHOD OF TAKING SAMPLES

To obtain relatively large and aseptic samples of meat such as were used in these experiments is not an easy matter, and extreme care had to be taken to prevent bacterial contamination. After several failures samples free from bacteria were obtained in the following manner: At the laboratory the hindquarter was transferred at once to a special inoculating room about 10 feet square. The walls and floors of this room had been previously washed with the mercuric-chlorid solution. A special canopy ceiling consisting of cheesecloth tacked on a light frame had been placed in the room at the height of about 10 feet, and this was sprayed with a solution of liquor cresolis compositus just before taking the samples. The floor and walls were also sprayed at the same time with the compound cresol solution and were damp while the samples were being taken, the idea being to have the floor, walls, and ceiling moist, so that any floating dust particles would stick to them.

For taking the samples a number of large, heavy-bladed scalpels and long dissecting forceps were used; these had been sterilized and wrapped in cotton. Large plugs of meat, approximating 3-inch to 4-inch cubes, were cut from the muscular tissue, avoiding connective tissue and fat as much as possible. These plugs, weighing from 274 to 512 gm., the average being 377 gm., were immediately transferred to sterile crystallizing dishes fitted with deep glass covers. In cutting out the plugs the line of incision was first thoroughly seared with a hot spatula. Then a light cut was made through the outside to the depth of about 0.5 cm. and the knife used for the incision was laid aside. A second sterile knife was then used for continuing the deeper incision. This was done in order not to carry in any of the mercuric-chlorid solution which might have adhered to the outside. The outer or exposed portions of the meat samples were always trimmed away to the depth of at least half an inch in order to eliminate those portions which had come in contact with the bichlorid gauze. Thirty-three samples were taken in this manner.

The dishes containing the samples were weighed, the covers sealed with adhesive tape, and over the tape were placed strips of tin foil. This was done for the double purpose of preventing evaporation and the possibility of bacterial contamination from the outside.

### BACTERIOLOGICAL CONTROL OF SAMPLES

The dishes containing the meat samples were placed in the incubator and carefully watched from day to day for evidence of bacterial growth.

Twenty-four of the thirty-three samples showed bacterial contamination upon incubation—that is, visible bacterial growths developed on

the moist surface of the samples, which furnished a good culture medium for bacterial growth. These samples were, of course, rejected. The remaining samples showed no visible bacterial growths upon incubation and were removed from the incubator one at a time after intervals ranging from 7 to 100 days and subjected to a bacteriological examination.

In examining the samples bacteriologically, aerobic and anaerobic cultures were first made from the exuded juice. With sterile instruments bits of muscular tissue were then cut from the outside of the samples and used for cultures. The samples, which, as before stated, consisted of large rectangular pieces approximating 3-inch cubes, were then cut in two with sterile instruments and cultures made by taking bits of the muscular tissue from the center of the samples. A half dozen or more cultures were taken from each sample. Smear preparations were also made from the exuded juice and from the outer and inner portions of the samples and were stained for bacteria.

Upon bacteriological examination nine of the samples were passed as sterile, there being no growths in any of the cultures made from these samples and the smear preparations being negative. These samples were then subjected to chemical analysis (Table I).

The fact that so large a proportion of the samples, 24 out of 33, or about 72 per cent, developed bacterial growths goes to show how difficult it is to obtain sterile samples of meat.

### METHODS OF CHEMICAL ANALYSIS

After having taken the samples of muscular tissue for incubation the remainder of the quarter of beef was placed in cold storage at 33° F. for 17 hours, when a composite sample of the lean meat was taken for analysis. Analytical work was started about 24 hours after the slaughter of the animal.

All samples of meat, both fresh and after incubation, were finely ground, placed in glass jars, tightly sealed, and analytical work was started promptly.

Moisture and fat determinations were made on all samples. Moisture was determined by drying the material in vacuo over sulphuric acid, and fat was determined in the dry residue by extraction with ether.

PREPARATION OF EXTRACT.—A 0.9 per cent solution of sodium chlorid, saturated with thymol, was used as a solvent. One hundred gm. of the finely ground tissue were macerated in a mortar with the salt solution until a mixture of uniform consistency was obtained. The material was then transferred to a 2-liter volumetric flask, made to volume with the salt solution, and shaken at intervals during a total extraction period of 24 hours. The mixture was then filtered and analytical work begun immediately. Extractions were made in duplicate and the work was carried on in a refrigerated room at a temperature of about 35° F.

ACIDITY was determined by titrating 50 c. c. of the filtered extract against standard sodium-hydroxid solution, using phenolphthalein as an indicator. The results are calculated in terms of lactic acid.

TOTAL CREATININ was determined according to the method of Folin as modified by Emmett and Grindley (1, p. 515). The results are calculated in terms of creatinin.

FREE CREATININ was determined essentially according to the method of Folin (2). Standard creatinin solutions were made from creatinin which had been standardized against *N/2* potassium bichromate. With close attention to all details this method was found to give very satisfactory results.

Table I shows the changes in the creatin and creatinin content of lean beef autolyzed under aseptic conditions for periods ranging from 7 to 100 days.

TABLE I.—*Changes in creatin and creatinin content of muscle during aseptic autolysis at 37° C.*

| Serial No. | Incubation period. | Percentage of acid as lactic. | Percentage of total creatinin. | Percentage of free creatinin. | Percentage of creatin calculated as creatinin. | Percentage of total creatinin as free creatinin. |
|---|---|---|---|---|---|---|
| | Days. | | | | | |
| 109 | ..... | 3.15 | 1.73 | 0.036 | 1.694 | 2.08 |
| 110 | 7 | 3.03 | 1.97 | .422 | 1.548 | 21.42 |
| 111 | 14 | 3.15 | 1.91 | .603 | 1.307 | 31.57 |
| 112 | 21 | 3.16 | 1.91 | .706 | 1.204 | 36.96 |
| 113 | 28 | 4.75 | 1.63 | .756 | .774 | 46.38 |
| 120 | 42 | 4.33 | 1.64 | .761 | .879 | 46.40 |
| 121 | 64 | 4.53 | 1.55 | .670 | .880 | 43.23 |
| 122 | 77 | 5.02 | 1.62 | .742 | .878 | 45.80 |
| 124 | 93 | 4.74 | 1.54 | .707 | .833 | 45.91 |
| 125 | 100 | 4.76 | 1.68 | .728 | .952 | 43.33 |

Changes in total creatinin are fairly marked. Samples incubated for 7, 14, and 21 days show increases in total creatinin amounting to 0.24, 0.18, and 0.18 per cent, respectively. Samples incubated for longer periods, ranging from 28 to 100 days, show appreciable losses in total creatinin varying from 0.19 to 0.05 per cent. On the whole, these data show first an increase in total creatinin and later a decrease, the increases being somewhat larger than the decreases.

The changes in the free creatinin are very marked. The fresh material contains 0.036 per cent of free creatinin, while the sample incubated 7 days contains 0.422 per cent, an actual increase of 0.386 per cent, or a relative increase of 1,722 per cent. Samples incubated for 14, 21, 28, and 42 days show further increases in free creatinin, but the rate of increase is less rapid with each succeeding period. The maximum percentage of free creatinin, amounting to 0.761 per cent, is found in case of the sample incubated 42 days. This is an actual increase of 0.725 per

cent of creatinin as compared with the fresh material. Samples incubated for periods ranging from 64 to 100 days show slight and irregular decreases in free creatinin as compared with the sample incubated 42 days.

The creatin content of the samples, which is calculated by subtracting the percentage of free creatinin from that of total creatinin, shows decreases which correspond to the increases in free creatinin.

The relation between the free creatinin and total creatinin is of special interest. The fresh material contains 2.08 per cent of the total creatinin in the form of free creatinin, while in case of the sample incubated for 7 days the percentage has increased to 21.42. The increases in succeeding periods are less rapid, until a maximum increase is reached in case of the sample incubated 42 days, which contains 46.40 per cent of the total creatinin in the form of free creatinin. However, practically the maximum increase is reached in case of the sample incubated 28 days in which 46.38 per cent of the total creatinin is in the form of free creatinin.

These results show that under the conditions of the experiment an equilibrium is established between the creatinin and creatin. These findings confirm in a remarkable degree results obtained by Myers and Fine (5) in their work with pure solutions of creatin and of creatinin. They incubated solutions of the individual bases for a total period of 337 days, and determined free and total creatinin in each of the solutions at intervals. In case of the solution of creatin, it was found that there was a gradual change of creatin into creatinin until at the end of the period an equilibrium had been established with 44.45 per cent of the total creatinin in the form of free creatinin. In case of the solution of creatinin the change was in the other direction, there being a decrease in creatinin and an increase in creatin, until at the end of 337 days an equilibrium had been established with the relative proportions of creatin and creatinin identical with those noted above.

It is not to be inferred from these findings that the changes which took place in the creatin and creatinin content of muscular tissue during autolysis are entirely natural changes of one base into the other. In case of the autolytic experiments with muscle, practically the maximum change of creatin into creatinin had taken place at the end of 28 days, and nearly half of the total change had taken place in 7 days.

In Myers and Fine's experiments (5) with a solution of pure creatin only 9 per cent of the total creatinin was present in the form of free creatinin at the end of 13 days, and after 53 days only 29 per cent. In our autolytic experiments with muscle, on the other hand, 25.41 per cent of the total creatinin was in the form of free creatinin at the end of 7 days, and 46.31 per cent at the end of 28 days.

It is very evident that the rate of change of creatin into creatinin during the autolysis of beef muscle was greatly accelerated by some agent. The acids in the meat may have facilitated the change in some degree;

but the facts seem to indicate that in considerable part, at least, the change of creatin into creatinin during the autolysis of beef muscle was caused by enzym action.

### ANTISEPTIC AUTOLYSIS EXPERIMENTS

Muscular tissue, consisting of the pillar of the diaphragm, was obtained from the carcass of a steer immediately after slaughter. The meat was freed from visible fat and connective tissue and finely ground. Thirty-five gm. of meat were weighed into a mortar with 20 gm. of sand, and 50 c. c. of a 0.9 per cent solution of sodium chlorid were added. The tissue was ground to a mass of uniform consistency and then transferred to a 250 c. c. Erlenmyer flask with the aid of 100 c. c. of the salt solution, and the flask was stoppered with a rubber stopper. Sixteen samples were prepared in this manner. After all the samples had been prepared, 2 c. c. each of chloroform and toluol were added to each flask which was then thoroughly shaken. Fourteen of the flasks were then placed in an incubator where they were held at 37° C. for various periods of time. The flasks were shaken daily to insure saturation of the solutions with the antiseptics. Two flasks were placed in a cold-storage room at a temperature of 34° F. and shaken at intervals for a period of 24 hours for the purpose of determining the creatin and creatinin in the fresh material.

### BACTERIOLOGICAL CONTROL OF SAMPLES

Before adding the antiseptics and before incubation, bacterial counts were made of three of the samples, Nos. 2, 8, and 17, which had been prepared as described above. In making the counts, 0.5 c. c. and 1 c. c. portions of the samples were withdrawn with sterile pipettes and added to tubes of melted agar which were immediately poured into Petri dishes and incubated. The bacterial counts on the three samples were as follows:

Sample 2.....2,116 bacteria per cubic centimeter.
Sample 8.....1,480 bacteria per cubic centimeter.
Sample 17.....1,584 bacteria per cubic centimeter.

The samples were prepared one at a time in the order in which they were numbered—that is, from 1 to 18—and the higher bacterial count in the case of sample 2 is probably due to the fact that this flask was the first one prepared and remained standing for several hours at room temperature, thus giving time for bacterial multiplication before the counts were made. The three counts were made in order to give some idea of the average number of bacteria in the samples before adding the antiseptics and before incubation.

The samples were removed from the incubator for chemical analysis at the intervals given in Table II. In testing the samples bacteriologically, two portions of 1 c. c. each were removed with sterile pipettes and

agar plates made therefrom. In withdrawing the portions for cultures the point of the pipette was introduced well below the surface of the liquid so as to avoid drawing up any of the toluol which floated on the surface. The chloroform, being heavy, settled to the bottom.

In order to avoid carrying over any of the toluol on the pipettes, the ends of the pipettes were washed with sterile, distilled water before their contents were delivered into the agar tubes. A single colony was observed in one of the plates made on the fourth day and single colonies were observed in each of the plates made on the eighth day, but after this the plates remained sterile.

The absence of bacterial development in the plates may have been due to inhibition of growth by small amounts of the antiseptics dissolved in the meat infusion rather than to actual destruction of the organisms present. However, the results seem to afford ample evidence that there was no bacterial multiplication in the samples during the course of the experiment.

## CHEMICAL STUDIES

Moisture was determined in the fresh material for the purpose of correcting for the volume of water in the meat.

Creatin and creatinin were determined in the filtered extracts from the various samples according to the methods of Folin, as previously noted (2).

Table II shows the changes in the creatin and creatinin content of muscular tissue from the ox incubated at 37° C. in the presence of antiseptics for periods ranging from 2 to 84 days.

TABLE II.—*Changes in creatin and creatinin content of beef muscle during antiseptic autolysis at 37° C.*

[Expressed as percentages of fresh material.]

| Serial No. | Incubation period. | Percentage of total creatinin. | Percentage of free creatinin. | Percentage of creatin calculated as creatinin. | Percentage of total creatinin as free creatinin. |
|---|---|---|---|---|---|
| 1 | | 0.28 | 0.0047 | 0.2753 | 1.67 |
| 2 | 48 hours | .29 | .0140 | .2760 | 4.78 |
| 4 | 95 hours | .28 | .0195 | .2605 | 7.07 |
| 5 | 7 days | .29 | .033 | .257 | 11.26 |
| 6 | 14 days | .29 | .049 | .241 | 16.65 |
| 7 | 21 days | .28 | .060 | .220 | 21.09 |
| 8 | 28 days | .27 | .067 | .203 | 25.09 |
| 9 | 35 days | .28 | .080 | .200 | 28.12 |
| 10 | 42 days | .28 | .107 | .173 | 37.61 |
| 11 | 49 days | .28 | .098 | .182 | 35.53 |
| 12 | 56 days | .28 | .101 | .179 | 36.61 |
| 13 | 63 days | .29 | .109 | .181 | 37.19 |
| 14 | 70 days | .29 | .112 | .178 | 38.21 |
| 15 | 77 days | .28 | .113 | .167 | 39.70 |
| 17 | 84 days | .29 | .113 | .177 | 38.53 |

There are practically no changes in the total creatinin in contrast to the fairly marked changes in this constituent noted in case of the aseptic autolytic experiment. This fact does not indicate that the changes in total creatinin content observed in case of the aseptic autolytic experiment are in error, but rather that the antiseptics used in the second experiment probably prevented the change.

There is a marked increase in free creatinin during the course of the experiment, the increase taking place most rapidly in the early stages of the incubation period, and less rapidly toward the end of the experiment, until finally there was practically no change.

On account of the different bases of calculation, these data can not be compared directly with similar data obtained in case of the aseptic autolytic experiment. However, the general trend of the change in free creatinin is the same in each experiment. In the first experiment a maximum production of free creatinin was reached in 42 days, in the second experiment in 84 days.

Creatin shows decreases corresponding to the increases in creatinin. The data showing changes in the relation of free creatinin to total creatinin indicate most clearly the changes in these constituents during the course of the experiment. The transformation of creatin into creatinin takes place most rapidly during the first 24 hours, and the rate of change steadily decreases during the course of the experiment, until at the end of 77 days a maximum change is reached, the free creatinin then constituting 39.70 per cent of the total creatinin. It is possible that if the experiment had been continued for a much longer time a larger proportion of creatin would have been converted into creatinin. These data confirm the changes of creatin to creatinin observed in the case of the aseptic autolytic experiment, and also the fact that the total extent of the change is limited. In the first experiment a maximum change of 46.40 per cent of total creatin, calculated as creatinin, to creatinin was observed, while in the second experiment the total change amounted to 39.70 per cent.

The results obtained in the antiseptic autolytic experiment confirm those obtained in the experiment conducted under aseptic conditions, both as regards change of creatin into creatinin, and in that the total extent of the reaction is limited, but do not confirm those showing first an increase and later a decrease in total creatinin.

## DISCUSSION OF RESULTS

The results of the experiments reported in this paper show very clearly the transformation of muscle creatin into creatinin during autolysis. To a very considerable degree this transformation must be regarded as due to the action of enzyms.

These findings are substantiated by the work of Gottlieb and Stangassinger (3), Stangassinger (9), Rothmann (7), Rowe (8), and Myers and

Fine (5). Mellanby (4) obtained contrary results; but, as has already been noted, a careful examination of his paper indicates something wrong with his work, since he was unable to detect creatinin under conditions in which it was undoubtedly present. His findings should not be taken too seriously. The ability of autolyzing muscular tissue, as well as of other body tissues, to transform creatin into creatinin seems to be quite clearly established.

In the aseptic autolytic experiment there was first an increase in total creatinin and later a decrease as compared with the amount present in the fresh material, while in the experiment carried on under antiseptic conditions there was practically no change. As has been previously noted, it does not follow that the results of the first experiment are in error, but it is possible that in the second experiment the presence of antiseptics prevented these changes in creatin.

A brief examination of the work of previous investigators on this point may throw some light on the question. Gottlieb and Stangassinger (3) observed at first an increase and later a decrease in the total creatinin content of muscular and other body tissues and fluids on autolysis. Stangassinger (9) found an increase in the total creatinin content of blood and liver of dogs and later a decrease in the total creatinin content of the liver. Rothmann (7) found that extracts of the liver and kidney of dogs destroyed added creatinin in a marked degree, and that there was a marked increase in the creatin content of the portal blood of a dog. Rowe (8) observed that extracts from the parathyroid and adrenal glands of sheep destroyed added creatin. Pekelharing and van Hoogenhuyze (6) found an increase in the creatin content of the muscles of dogs after rigor mortis and heat rigor. Myers and Fine (5) found an increase in the total creatinin content of autolyzing human blood and rabbit liver, and a decrease in the total creatinin content of dog muscle. On the whole, the work of these investigators confirms our findings concerning changes in the total creatinin content of beef muscle during aseptic autolysis.

In keeping with the results obtained by Pekelharing and van Hoogenhuyze (6) concerning the effects of rigor mortis upon the creatin content of muscular tissue, it seems very probable that the increase in the total creatinin content of the muscle in our aseptic autolytic experiment was due to the changes accompanying rigor mortis. While analytical work was started 24 hours after the slaughter of the animal, at which time rigor was assumed to be complete, yet in a study of the effects of autolysis upon the soluble muscle proteins changes were observed which indicated that it was not complete at that time.

The establishment of an equilibrium between creatin and creatinin in solutions of the individual bases, as observed by Myers and Fine (5), and our finding as to the establishment of a similar relation between creatin and creatinin in autolyzing muscular tissue, is a matter of more

than passing importance. It denotes, first, that creatin is readily converted into creatinin in pure solution, and, second, that in autolyzing muscular tissue the rate of reaction is very greatly accelerated, but that the total extent of the change is the same in either case. The more rapid change of creatin into creatinin in the autolyzing tissue may safely be assumed to be due, in large part, at least, to enzym action. This conforms to our idea as to the catalytic nature of enzyms. The gradually reduced rate of change of creatin to creatinin during autolysis is in conformity with the law of mass action. These observations are of more interest as regards the chemical relationship of the two substances than on account of their physiological relationship, since in the animal body any change of creatin to creatinin is accompanied with the rapid removal of the creatinin, so that, so far as this factor is concerned, the change always takes place at its maximum velocity. The clear establishment of the fact that muscular tissue has the power in a marked degree of converting creatin into creatinin must be regarded as having an important bearing upon the formation of creatinin in the body. Without going into a discussion of other investigations bearing upon this subject, it may be said that there is much evidence in support of the theory that muscle creatin is the source of urinary creatinin, with a creatin-creatinin-free diet, and considerable evidence to the effect that in part, at least, the transformation of creatin into creatinin takes place in the muscular tissue.

## SUMMARY

The results of the investigations reported in this paper concerning the effects of autolysis upon the creatin and creatinin content of muscular tissue of the ox may be summarized as follows:

(1) Muscular tissue has in a marked degree the property of converting creatin into creatinin.

(2) In the course of autolysis an equilibrium is finally established between creatin and creatinin.

(3) Muscular tissue appears to have in an appreciable degree the ability both to produce and to destroy creatinin.

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# STORAGE-ROTS OF ECONOMIC AROIDS

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## INTRODUCTION [1]

The economic aroids within the scope of this article include various species and varieties of the genus Colocasia obtained from numerous warm regions throughout the world; a species of Alocasia received from Dutch Guiana under the varietal name "Eksi-taya" and *Xanthosoma sagittifolium* (L.) Schott, a native tropical American species. These plants are all of greater or less importance for human food in many tropical and subtropical countries, and they are being grown commercially or experimentally in the southern United States.

The Trinidad dasheen, a variety of taro, gives the greatest promise of success in the United States. It differs from many other taros in that it produces a considerable number of cormels, or "tubers," [2] of edible size, in addition to the large, edible, central corm. There are a number of varieties resembling it more or less closely. China is believed to have been the original home of the Trinidad dasheen, which is referred to *Colocasia esculenta* (L.) Schott.

Another group of taros, resembling the Trinidad dasheen in general leaf and floral characters and in the production of a large number of tubers, is represented by the Yu-to variety, from Mukden, Manchuria. Several of the Japanese taros, or "imos," are similar to this variety. The tubers are often very numerous, but usually quite small. These varieties are at present also referred to *C. esculenta*.

The Egyptian taro, called "Qolqas," is a member of another group of taros probably belonging to *C. antiquorum* (L.) Schott. A variety of this type, obtained by the Department from Cat Island, S. C., in 1906, is representative of this group. This group is distinguished from the

[1] The first four paragraphs of the introduction were prepared by Mr. R. A. Young, of the Office of Foreign Seed and Plant Introduction, Department of Agriculture.

[2] The word "tuber," the commercial term for "cormel" in the case of the dasheen, is used instead of "cormel" in this paper.

preceding by having a spathe that opens broadly, as well as by the general aspects of the plants. *C. indica* (Lour.) Kunth, a native of Java, was also used in these investigations.

The storage of dasheens by piling the tubers and corms in the field and overlaying them with straw and earth fully protects them against freezes and yields itself readily in other respects to a successful handling of the crop. In these piles, however, unless special means of ventilation are provided, many of the tubers and corms rot so badly as to render them useless for food or propagation. From such decayed material a considerable variety of organisms was isolated during the winter of 1912 and 1913. From similar material about the same organisms were isolated the following year. With these organisms inoculation experiments were made during the winter of 1913 and 1914, and repeated again in 1914 and 1915. Out of the different organisms isolated four were found to be wound parasites under certain conditions. Macroscopically it is not always easy to distinguish the different rots, since in some cases more than one of the rot-producing organisms may be present. An accurate diagnosis is also frequently obscured or rendered difficult by the invasion of saprophytic bacteria and fungi. Furthermore, the striking similarity of some of the rots in the earlier stages renders a diagnosis extremely difficult. While the writer can usually distinguish macroscopically typical cases of the several rots in the later stages, the only sure method is the preparation of cultures.

## JAVA BLACKROT

The most common and destructive of the storage-rots is called the "Java blackrot" because of its resemblance to the Java blackrot of the sweet potato (*Ipomoea batatas*) caused by the same organism, *Diplodia tubericola*. The causal fungus has been isolated repeatedly during a period of three years from a number of varieties. This disease is particularly interesting in view of the fact that different species of the genus Diplodia obtained from other hosts widely separated botanically from the dasheen will cause a decay of the latter identical in character.

## DESCRIPTION OF JAVA BLACKROT

The tissue when first invaded by the fungus is but little or not at all changed in color and is soft, slimy, and stringy. The substance of the corm or tuber becomes pasty and will, if picked up by the forceps, draw out in a threadlike manner. It is often difficult to distinguish the decay caused by the blackrot fungus in the early stages from the decay produced in the initial stages by other organisms without resorting to plate isolations. A little later, however, the tissue becomes slightly pinkish and then gradually turns black, and in this respect differs from the decayed tissue produced by the other organisms. At the same time the rotted portion of the tuber gradually becomes firmer by the escape of moisture.

Plate LXXXI, figures 1 and 2, shows the typical rots of *C. esculenta* and *Alocasia* sp., respectively, produced by the Java blackrot fungus.

The rot progresses slowly. About seven days elapse after inoculation before any noticeable softening of the tuber occurs under optimum conditions and about four to eight weeks are required for complete destruction of the tuber and blackening of the tissue. Finally both the tubers and corms become very dry and hard and are cut by a knife with difficulty. The middle lamella is first dissolved, the hyphæ later penetrating the cell walls and burying themselves among the starch grains. The tissue finally becomes a disorganized mass and powdery when completely dried. Under normal conditions the rot does not produce any, or, at most, only slight shrinking or malformation of the tuber. In fact, a whole tuber may be completely destroyed internally and become black throughout without much external evidence of it. Fruiting bodies later develop, but they are mostly covered by the epidermis and can scarcely be detected without rupturing the surface.

Under natural conditions the corms decay more readily than the tubers, although the latter are frequently met with in storage and succumb easily to artificial inoculation. It is evident from a careful study of material that natural infection originates in the wounds made by breaking the tubers from the corms and at points where the roots are broken off. After becoming established the fungus may spread in all directions without penetrating deeply until the surface of the corm is well covered, and then it may penetrate farther in; or it may cover an area 1 or 2 inches in diameter and push inward to the center in the form of a cylinder.

## CAUSE OF BLACKROT

The writer has isolated and successfully inoculated into the dasheen species of Diplodia from five different hosts, as follows: *D. tubericola* (E. and E.) Taub. from sweet potato; *D. gossypina* Cke. from a dead limb of cotton; *D. maclurae* Speg. from a dead branch of *Toxylon pomiferum* Raf. from New Jersey; *Diplodia* sp. from a limb of *Mangifera indica* from Cuba, furnished by Dr. J. R. Johnston, pathologist of the Cuban Experiment Station, and a species of Diplodia from dasheen which, because of its great similarity to *D. tubericola*, is referred to that species. The type of decay produced by these different species is macroscopically the same. It is a well-known fact that there are a great number of different species of Diplodia described in the literature, many of which may prove to be identical. No attempt has been made to go into the taxonomy of this group, but it may be of interest to note the points of similarity and difference between the species here studied. The organism isolated from dasheen can not be distinguished in culture from *D. tubericola* from sweet potato. Both develop into stroma in culture and on the host, and the spores differ but little in shape (fig. 1, *A*, *B*) and size.

*D. gossypina* has been shown by Edgerton (3) to be primarily a wound parasite of cotton bolls and by Taubenhaus (9) [1] to produce a

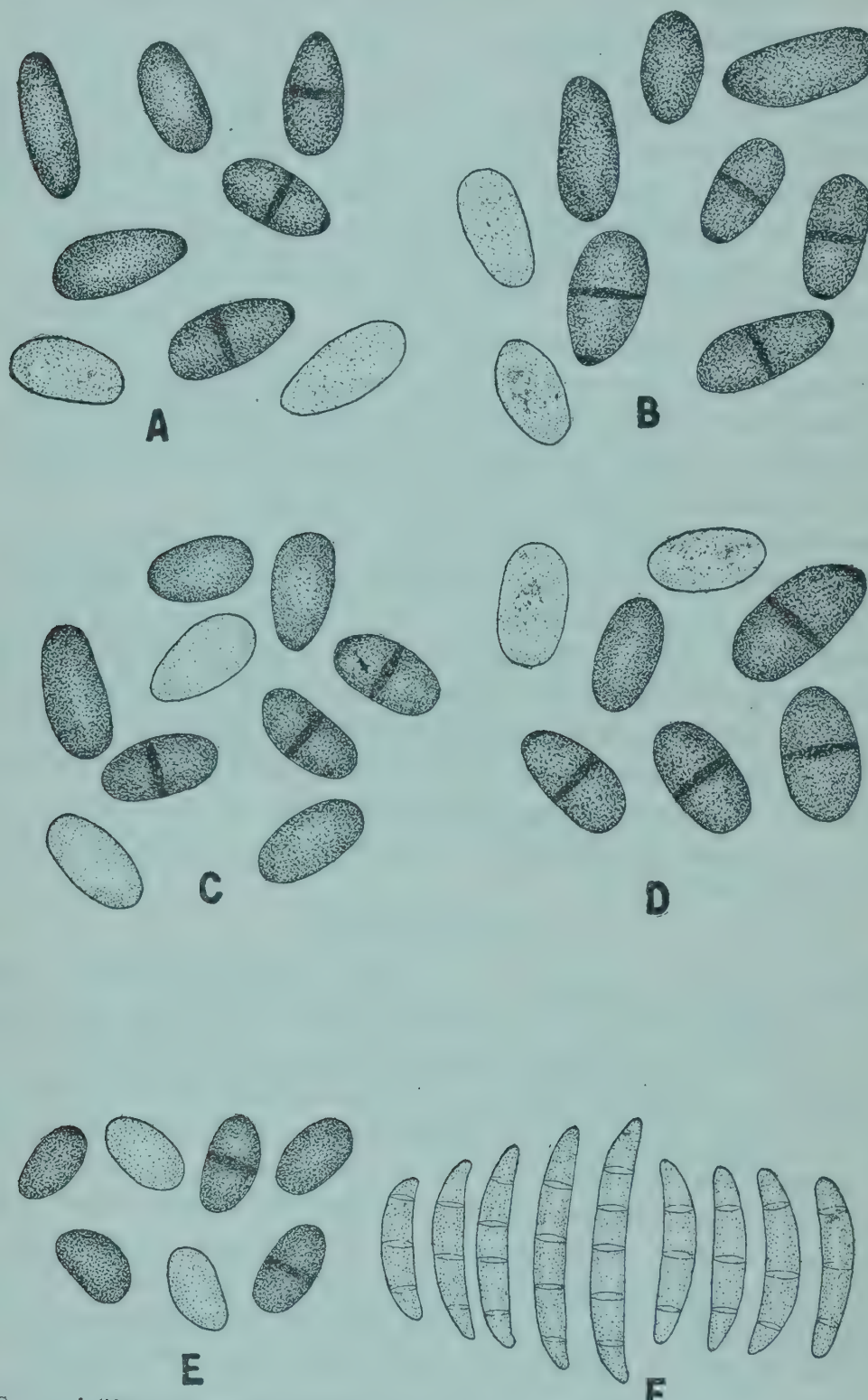


FIG. 1.—Spores of different storage-rot organisms: *A*, *Diplodia tubericola* from dasheen; *B*, *Diplodia tubericola* from sweet potato; *C*, *Diplodia gossypina* from cotton; *D*, *Diplodia* sp. from *Mangifera indica*; *E*, *Diplodia maclurae* from *Toxylon pomiferum*; *F*, *Fusarium solani*. × 500.

typical Java blackrot of sweet potatoes. The writer found the fungus produced a decay of dasheens identical with that caused by *D. tuberi-*

[1] Reference is made by number to "Literature cited," p. 571.

*cola* from sweet potatoes and from dasheens and to agree closely in cultural characteristics and in shape (fig. 1, *C*) and size of spores. While *Diplodia* sp. from *Mangifera indica* and *D. maclurae* both produce a typical rot of dasheens and agree with the other species in cultural characteristics and shape of the spores (fig. 1, *D*, *E*), the spores of the latter fungus are uniformly smaller in size. *D. maclurae* is less virulent for dasheens than the other species. The spores from the host of the different species studied measure as follows:

*Diplodia tubericola* from sweet potato, 22.3 to 34.4 by 10.3 to 13.7μ. Average, 11.6 by 25.5μ (30 measurements).

*Diplodia tubericola* from dasheen, 22 to 33 by 10.3 to 13.7μ. Average, 11.3 by 26.5μ (30 measurements).

*Diplodia gossypina* from cotton, 20.1 to 28 by 9 to 13.4μ. Average, 11.5 by 24.5μ (31 measurements).

*Diplodia maclurae* from *Toxylon pomiferum*, 17.5 to 22.3 by 8.5 to 11μ. Average, 9.7 by 19.7μ (30 measurements).

*Diplodia* sp. from *Mangifera indica*, 23 to 31.6 by 12 to 14.1μ. Average, 13 by 26.2μ (31 measurements).

In 1906 Charles (2) isolated and studied a species of Lasiodiplodia from the fruit of *Mangifera indica*, but left the question unsettled as to whether it was the same organism found on the sweet potato. However, the results obtained by the writer by inoculation studies with the above species and by Taubenhaus (9), who obtained positive infections of sweet potatoes with several species of Diplodia, suggest the possible identity of many of these forms described as different species. The results also indicate that these crops are exposed to infection from several sources.

## INOCULATION EXPERIMENTS

### INOCULATION OF COLOCASIA ESCULENTA

On January 6, 1914, thirteen dasheen tubers, after being thoroughly washed and disinfected for 10 minutes in mercuric chlorid (1:1,000) and rinsed in water, were inoculated in a wound at the end by inserting spores and hyphæ of *D. tubericola* from dasheen. All inoculations were made from cultures grown on cooked potato cylinders in which spores were present, although in many cases they were hyalin and nonseptate. After inoculation the tubers were placed in a large, uncovered, moist chamber and subjected to the temperature and humidity of the laboratory room. By January 19 the rot had noticeably started on all the tubers, and by January 28 all were completely decayed. The causal organism was recovered in pure culture from each tuber. The checks, six in number, similarly located remained healthy.

On the same date seven tubers prepared as above and inoculated with the same organism were placed in a covered moist chamber with wet filter paper in the bottom and placed on a shelf in the laboratory. These

tubers were kept under observation until February 17, and none showed any evidence of decay. The checks, two in number, under similar conditions but not inoculated, remained healthy. This and subsequent experiments showed that better results could be obtained by merely exposing the inoculated tubers to the surroundings of the laboratory room. The use of moist chambers, therefore, was abandoned, with the exception of an occasional trial experiment to be noted later. Disinfection likewise was no longer practiced, since the tubers were immediately exposed to reinfection from the air of the room. Although the rot caused by *D. tubericola* is very easily recognized and characteristic when once known, cultures were made from nearly all the decayed tubers, in order to be sure the rot was caused by the used organism. The influence of temperature and moisture on these storage rots will be discussed later.

On January 16, 1914, four tubers were inoculated in the usual way with *D. tubericola* from dasheen. By February 18 all were rotted and the causal organism recovered in pure culture. The checks, two in number, remained healthy.

On March 1, 1914, twelve tubers were inoculated with *D. tubericola* from dasheen. On March 12 several tubers showed evidence of decay and by March 20 nine were partially rotted. A portion of some of the tubers was black, and pycnidia containing hyalin 1-celled spores were present. On June 1 all the tubers were completely decayed. The checks, five in number, remained sound.

On January 14, 1915, four tubers were inoculated with *D. tubericola* from sweet potato. On February 18 all the tubers were rotted, and the causal organism was recovered in pure culture. Two days later ten tubers were inoculated and divided into two equal lots, one being placed in an incubator, the temperature of which varied from 34° to 35° C., and the other in an ice box, the temperature of which varied from 12° to 13°. By February 3 all the tubers in the incubator were rotted and the causal organism was recovered in pure culture, while those in the ice box and the five checks remained sound.

On March 26 six tubers were inoculated with *D. maclurae*. Some time later one was completely decayed and yielded *D. maclurae* in culture; the others remained sound. Four more tubers were inoculated on May 13, 1915, and on June 6 three tubers were half-decayed, *D. maclurae* being recovered from two, *Rhizopus nigricans* from one, and *Fusarium* sp. from one. The checks, five in number, remained sound.

Six other tubers were inoculated on May 20, 1915. On June 1 two were completely decayed and four remained sound.

On December 23, 1914, nine tubers were inoculated with *D. gossypina*, five of which were placed in an open receptacle on the laboratory shelf and four in a moist chamber. All the exposed tubers were rotted on

January 13, and *D. gossypina* was recovered. In the moist chamber two tubers were sound; the other two rotted a very little, one of which yielded *Fusarium solani* and the other *F. oxysporum*. Out of six other tubers inoculated on March 1 five were completely rotted on March 26. The checks, five in all, remained sound.

On January 29, 1915, ten tubers were inoculated with *Diplodia* sp. from *Mangifera indica*. Nine of these tubers showed evidence of rot on February 8; and on February 20 six were completely decayed, three were half-decayed, and one remained sound. The checks, five in number, remained sound.

### INOCULATIONS OF XANTHOSOMA SAGITTIFOLIUM

On November 30, 1914, five tubers were inoculated with *Diplodia tubericola* from dasheen and five with the same organism from sweet potato. On December 23 all the tubers in both lots were rotted and the causal organism was recovered in pure culture. The checks, five in number, remained sound. Six other tubers were inoculated on December 9 with the sweet-potato organism, and on January 2, 1915, *D. tubericola* was recovered from four and *Fusarium oxysporum* from two.

On January 4, 1915, ten tubers were inoculated with *D. maclurae* and five with *D. gossypina*. By February 10 five of the former and three of the latter were decayed and the causal organisms recovered. The five checks remained sound.

### INOCULATION OF COLOCASIA INDICA

On December 9, 1914, four tubers were inoculated with *D. tubericola* from dasheen and five with the same organism from sweet potato. All the tubers in both lots were completely decayed on January 2, 1915, and the causal organism was recovered. The checks, four in number, remained sound.

### INOCULATION OF ALOCASIA SP.

Five tubers were inoculated on January 2 with *D. tubericola* from dasheen, and on February 10 three were decayed. *D. tubericola* was recovered from two and *Fusarium* sp. from one. The two others and the five checks remained sound. On the same day five tubers were inoculated with the same organism from sweet potato, and on February 10 one tuber was sound. The four others were only partially decayed, but *D. tubericola* was recovered from the rotted portion. It appears that, while this species is not wholly immune to the rot, it is more resistant than the others. On January 4, 1915, four tubers were inoculated with *D. maclurae* and four with *D. gossypina*. All those inoculated with *D. maclurae* remained sound, but of those inoculated with *D. gossypina* two were completely decayed and two one-third rotted. The causal organism was recovered from each.

Table I gives the results of these inoculation experiments with *Diplodia* spp.

TABLE I.—*Results of the inoculations of tubers of Colocasia esculenta, Xanthosoma sagittifolium, C. indica, and Alocasia sp. with Diplodia tubericola, D. maclurae, D. gossypina, and Diplodia sp. from Mangifera indica*

| Organism. | *Colocasia esculenta.* | | | *Xanthosoma sagittifolium.* | | | *Colocasia indica.* | | | *Alocasia* sp. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inoculated. | Infected. | Checks. | Inoculated. | Infected. | Checks. | Inoculated. | Infected. | Checks. | Inoculated. | Infected. | Checks. |
| *D. tubericola* from dasheen | 36 | 29 | [a]13 | 5 | 5 | [a]5 | 4 | 4 | [a]4 | 5 | 5 | [a]5 |
| *D. tubericola* from sweet potato | 14 | 9 | 5 | 11 | 9 | 5 | 5 | 5 | 4 | 5 | 4 | .... |
| *D. maclurae* | 10 | 4 | 5 | 10 | 5 | 5 | .... | .... | .... | 4 | 0 | .... |
| *D. gossypina* | 15 | 10 | 5 | 5 | 3 | 5 | .... | .... | .... | 4 | 4 | .... |
| *Diplodia* sp. from *Mangifera indica* | 10 | 9 | 5 | .... | .... | .... | .... | .... | .... | .... | .... | .... |

[a] None of the checks became infected.

## POWDERY GRAYROT

Since this form of storage-rot has never been reported before, the writer proposes that it be known by the name "powdery grayrot." This, like many other common names of plant diseases, is somewhat misleading, since the rot in its early stages is soft and, if invaded by bacteria, is slimy on the surface. In the later stages, however, it becomes powdery and gray, this appearance serving to distinguish it from the other storage-rots.

### DESCRIPTION OF POWDERY GRAYROT

This rot has been isolated repeatedly from tubers and corms from Brooksville, Fla., and from specimens imported from Japan in May, 1915. Infection usually begins in the wounds made by breaking the tubers and corms apart, showing that it is probably strictly a wound parasite. When infected at such a point, the rot may spread rather widely over the surface, penetrating only half an inch or so; or it may penetrate under a small area to the center of the tuber or corm, though the number of specimens having been seen completely decayed by this organism is relatively small. In the final stages this rot becomes rather hard, dry, and powdery and is of a grayish color and crumbles when cut with a knife.

Numerous inoculation experiments have made it possible to study the progress of this rot more in detail in the laboratory. The first evidence of decay appears in 24 hours after inoculation on a cut surface, manifested by the formation of an ocherous to salmon-orange color. This color

becomes gradually darker and eventually turns brown, particularly just below the surface. Softening accompanied by stringiness of the tissue begins in 48 hours and extends to a depth of ¼ to ½ inch in one week. After a week or 10 days the surface becomes somewhat slimy and glistening from the production of pionnotes composed of numerous typical spores of the causal fungus. Upon drying, the specimen takes on a putty-like texture, shrinks perceptibly, and finally becomes dry and powdery and of a dark-grayish color. Plate LXXXII, figures 1 and 2, shows typical specimens of *Colocasia esculenta* and *Xanthosoma sagittifolium*, respectively, partially decayed by the powdery-grayrot fungus.

An examination of rotted material shows that the fungus first destroys the middle lamella and later to some extent invades the cells themselves, the tissue finally becoming a disorganized mass of separated cells.

## CAUSE OF POWDERY GRAYROT

For a period of three years *Fusarium solani* (Mart.) Sacc. has been repeatedly isolated in pure culture from decayed tubers and corms and has reproduced the characteristic rot when inoculated into dasheens. From such inoculated tubers the organism has been recovered and again made to produce the disease and subsequently recovered. The causal organism has been found to agree with *F. solani* as laid down by Appel and Wollenweber (1) both culturally and in size and septation (fig. 1, *F*) of spores, as shown by the following measurements: Tri-septate conidia taken from pionnotes of a 16-day-old culture on cooked Irish potato vary from 27 to 41 by 5.0 to 6.2$\mu$ and average 5.7 by 37.0$\mu$. Four-septate conidia, 34.4 to 51.6 by 5.2 to 6.2$\mu$, average 5.7 by 41.6$\mu$. Five-septate conidia, 5.4 to 5.9 by 41.3 to 51.6$\mu$, average 5.6 by 47.4$\mu$. In this connection it should be stated also that *F. solani* from Irish potato, isolated and identified by Wollenweber at Dahlem, near Berlin, Germany, produced a similar rot of dasheens. No difference between the two organisms could be detected either culturally or in their parasitic habits.

## INOCULATION EXPERIMENTS

A few preliminary experiments demonstrated that no decay would result when this fungus was spread on an unbroken surface. On the other hand, if placed on a freshly wounded surface, decay started in 24 to 48 hours, provided sufficient moisture was present to enable the fungus to get a start. These results seem to indicate that the fungus gains access to the tubers through wounds made by separating the tubers and corms or through wounds made by other means. The results of our experiments showed that two reliable methods of inoculation could be trusted; (1) Inoculation of the tuber by wounds made by pricking with a sterile needle or scalpel or (2) by splitting a corm or tuber in two and smearing spores on the cut surface. If the latter method was employed, a

film of water, such as may be supplied by a fine spray from an atomizer, must be provided for one or two days, after which the rot will continue independently. That *F. solani* smeared on a moist cut surface of dasheen develops as a wound parasite and not a saprophyte is evident from the fact that other fungi, such as *F. oxysporum* Schlecht., and *F. caudatum* Wollenw. isolated from dasheen, when similarly used produced no decay.

### INOCULATION OF COLOCASIA ESCULENTA FROM TRINIDAD

On January 21, 1915, six tubers were inoculated in a moist chamber by smearing spores of *F. solani* from dasheen on the cut surface. A soft-rot started in two days and by January 27 it had penetrated half an inch. The causal fungus was recovered from each. The checks, six in number, remained sound. On January 24, six tubers were inoculated and by February 11 all were completely decayed and *F. solani* was recovered from five. The plate in which the other planting was made was overrun with a species of Rhizopus. The checks, six in number, were sound. Six tubers inoculated on February 6 were completely rotted in 9 days. Six inoculations made on March 1, 1915, in the usual way were all rotted on March 9. No isolations were made. The checks, four in all, remained sound. On March 6 four tubers were inoculated into a cut surface at the end of the tuber and two on an unbroken surface at the side. Those inoculated into a wound rotted freely; the others remained sound.

On February 16 six tubers were inoculated with *F. solani* from Irish potato, and in nine days the tubers were well decayed, the rot being identical with that produced by tubers inoculated with the same organism from dasheen. The checks, six in all, remained sound. On March 1 six tubers were inoculated with *F. solani* from Irish potato, and in nine days all the tubers were mostly but not completely rotted. The four checks remained sound.

### INOCULATIONS OF COLOCASIA ESCULENTA FROM MANCHURIA

This is a variety of taro from Manchuria with small tubers about 2 inches long and 1 inch in diameter. On March 1 twelve of these tubers were inoculated with *F. solani* from dasheen. In seven days all the tubers were completely decayed, and the causal organism recovered from each. The checks, six in number, remained sound. An examination of the decayed specimens showed that, while the middle lamella was largely destroyed, the fungus did not to any extent invade the cells.

### INOCULATIONS OF XANTHOSOMA SAGITTIFOLIUM

On March 1 six tubers were inoculated with *F. solani* from dasheen. and in seven days the tubers were well decayed but not completely, the rot being identical with the rot of *Colocasia esculenta* produced by the same organism. *F. solani* was recovered from each tuber in pure culture. The checks, four in number, remained sound.

The results of these inoculation experiments with *F. solani* are given in Table II.

TABLE II.—*Result of the inoculations of Colocasia esculenta (Trinidad), C. esculenta (Manchuria), and Xanthosoma sagittifolium with Fusarium solani*

| *Colocasia esculenta* (Trinidad). | | | *Colocasia esculenta* (Manchuria). | | | *Xanthosoma sagittifolium.* | | |
|---|---|---|---|---|---|---|---|---|
| Inoculated. | Infected. | Checks. | Inoculated. | Infected. | Checks. | Inoculated. | Infected. | Checks. |
| [a]42 | 39 | [b]26 | 12 | 12 | [b]6 | 6 | 6 | [b]4 |

[a] Twelve tubers were inoculated with *F. solani* from Irish potato; all others with *F. solani* from dasheen.
[b] None of the checks became infected.

## SCLEROTIUM-ROT

The sclerotium-rot, while common in the storage heaps where high temperatures and a relatively high humidity prevails, is not so frequently met with under all circumstances as rots caused by *F. solani* and *D. tubericola*. The causal fungus is known to occur on a number of hosts widely separated in relationship, such as tomato (*Lycopersicon esculentum*), peanut (*Arachis hypogaea*), cabbage (*Brassica oleracea*), cotton (*Gossypium* spp.), violet (*Viola* spp.), and others. It has been found growing on the dead scales and other débris of many dasheen plants in the field in Florida, but not a single sure case has been found where it invaded the sound tissue. It, like the other fungi so far discussed, is primarily important only as a storage-rot.

### DESCRIPTION OF SCLEROTIUM-ROT

During a period of three years many tubers and corms have been examined which were somewhat mushy and watery and often covered by numerous almost spherical sclerotial bodies. The watery putrid condition often accompanying this decay is usually the result of saprophytic fungi and bacteria which followed the progress of the Sclerotium fungus. If this putrid substance is pared away, a firmer (Pl. LXXXI, fig. 3), almost odorless decay will be found from which a pure culture of the causal organism can be plated out. The rotted tissue is ocherous to brown in color, soft but not watery, with a tendency to stringiness. A sharp line characterized by a difference in color separates the healthy from the diseased tissue. The destruction of the tissue is apparently brought about by an enzym secreted by the fungus. At least there is a soft zone ¼ to ½ inch in width with the charactistic color of the rot from which the organism can not be isolated.

The hyphæ do not enter the cells to any extent, but the tissue finally becomes badly disorganized through the destruction of the middle lamella.

## CAUSE OF SCLEROTIUM-ROT

The sclerotium-rot is caused by *Sclerotium rolfsii* Sacc., a fungus which was first mentioned by Rolfs (6, p. 31) in 1893 and technically described by Saccardo (7, p. 257) in 1911.

In about seven days after inoculation in a moist chamber the sclerotial bodies begin forming. They are almost spherical, at first white, but later becoming brown, and finally nearly black, with a hard, shiny surface. This organism, the sclerotial bodies of which are composed of solid masses of fungus tissue, is, according to Wolf (10), parasitic on peanuts and a number of other legumes.

## INOCULATION EXPERIMENTS

All inoculations were made in moist chambers and kept in the laboratory except those in which temperature relations were studied, the results of which are discussed later. All attempts to produce the rot by placing bits of hyphæ on an unbroken surface of the tuber were unsuccessful. It was later found, however, that when the inoculations were made on a cut surface or in a small wound made by a scalpel they were uniformly successful if sufficient moisture was provided at the outset. Moisture was consequently furnished by spraying once or twice with water from an atomizer, and after 24 to 48 hours further applications of water were unnecessary. The fungus grows very rapidly and in a few days covers the whole surface of a tuber (Pl. LXXXII, fig. 3) split in two and even spreads onto the unwounded surface, although the scales of these aroids appear to be impenetrable by the fungus. Within a week the tissue is softened for half an inch or more, although under favorable conditions a month is often required to decay completely a tuber.

## INOCULATION OF COLOCASIA ESCULENTA

On January 14, 1915, six tubers of the Trinidad dasheen were inoculated with *S. rolfsii* by placing bits of hyphæ on a cut surface. Decay started in 2 days, and in 13 days the hyphæ had overrun the whole cut surface of the tuber and softened the tissue to the depth of half an inch. The checks, six in number, remained sound. On January 27 six tubers were inoculated, and by February 9 the tubers were well rotted and sclerotia forming. The checks, four in number, remained sound. On February 2 sixteen tubers were inoculated, and in 13 days all were softrotted and covered with a dense growth of hyphæ. The checks, five in all, remained sound. On February 6 four tubers were inoculated on an unbroken surface, but no growth had taken place by February 23, and they were thrown out. No checks. On February 15 eight tubers were inoculated and in 8 days they were all soft rotted, with sclerotia developing abundantly. Two tubers were inoculated on March 6 at the end in a small wound made by a scalpel and were well rotted by March 15.

INOCULATION OF XANTHOSOMA SAGITTIFOLIUM

On January 25 sixteen inoculations were made by placing bits of hyphæ on the cut surface and in 12 days all were softrotted and sclerotia abundantly produced. The six checks remained sound.

The results of the inoculation experiments with *S. rolfsii* are given in Table III.

TABLE III.—*Results of inoculation experiments with Sclerotium rolfsii*

| Host. | Date of inoculation. | Inoculated. | Infected. | Checks. | Checks infected. |
|---|---|---|---|---|---|
| *Colocasia esculenta* | Jan. 14 | 6 | 6 | 6 | 0 |
| Do | Jan. 27 | 6 | 6 | 4 | 0 |
| Do | Feb. 2 | 16 | 16 | 5 | 0 |
| Do | Feb. 6 | 4 | 0 | 0 | 0 |
| Do | Feb. 15 | 8 | 8 | 0 | 0 |
| Do | Mar. 6 | 2 | 2 | 0 | 0 |
| *Xanthosoma sagittifolium* | Jan. 25 | 16 | 16 | 6 | 0 |

SOFTROT

Many tubers have been examined which were softrotted and emitted a very disagreeable, repellent odor. At first the odor was supposed to be produced by saprophytic bacteria following the invasion of the host by some one of the organisms already discussed. From many such specimens, however, after paring away most of the rotted material, no fungi could be isolated. Microscopic examination of such material disclosed very actively motile bacteria which were readily isolated by the poured-plate method.

This is the only disease of the four studied which occurs to some extent in the field, mostly in the lower and poorly drained parts. Plate LXXXIII shows a corm and leaf attached as it appeared when lifted in the field. The lower part of the corm is decayed away. The organism isolated from this corm was used in some of the inoculation experiments which follow. The organism was also isolated from tubers and corms in the storage piles and once from the dark strands running through the corms. These strands sometimes appeared darker than normal, and microscopic examinations indicated invasion by some organism, but repeated attempts to isolate one failed until the winter of 1915, when a bacterium was isolated from a diseased strand in the center of a big corm by macerating bits of the decayed tissue in a tube of sterile water and pouring agar plates in the customary way. Numerous colonies later developed which proved to be identical with that produced by the other strains isolated from rotted tissue and to produce a rot similar to it. Usually these strands can be traced to the exterior of the corm, showing that the invading organism probably followed the strand. Under suit-

able conditions decay sets in which eventually results in the partial or complete destruction of the corm.

## DESCRIPTION OF SOFTROT

Softrot is characterized by being watery and slimy, with a disagreeable, repellent odor. The tissue is little or not at all changed in color under natural conditions. Under sterile, artificial conditions the surface becomes slightly reddish brown. Sections through diseased tissue show that the middle lamella is dissolved and the intercellular spaces are filled with bacteria. The cells themselves are seldom, if ever, invaded.

## CAUSE OF SOFTROT

The softrot of dasheen is caused by the well-known softrot organism of many vegetables, *Bacillus carotovorus* Jones. This conclusion was arrived at by a comparison in culture of the growth of the organism from dasheen with an authentic culture of *B. carotovorus* kindly furnished by Dr. L. R. Jones, of the University of Wisconsin, and by a series of cross-inoculations.

The comparison of growth of *B. carotovorus* on different culture media was made with three strains from dasheen as follows:

3624. *Bacillus carotovorus* Jones (furnished by Dr. Jones).
3595. A strain isolated from a partially softrotted Trinidad dasheen.
3616. A strain isolated from a Pat-long-fu taro (*C. esculenta*). (See Plate LXXXIII.)
3626. A strain isolated from the fibrovascular bundles at the center of a big corm of a Trinidad dasheen.

All these strains have produced the typical decay by inoculation. After rejuvenating the strains by transferring for several consecutive days to beef bouillon the following culture media were inoculated: Potato cylinders, milk, litmus milk, gelatin, nitrate solution, Cohn's solution, Dunham solution, Uschinsky's solution, beef bouillon, beef-agar slants, beef-agar plates and saccharose, lactose, dextrose, and glycerin bouillon in fermentation tubes. None of the strains grew in Cohn's solution. Gelatin was promptly liquefied by all strains, and nitrates were changed to nitrites when tested according to the method recommended by Smith (8).

Strain 3624 gave a prompt test for indol upon the addition of sulphuric acid and sodium nitrate, white strain 3595 yielded but a faint pink at first, which intensified upon warming to 75° C. The other two strains were doubtful. Strain 3624 was a slower grower than the others on practically all media as well as the less vigorous parasite, but the difference between the growths of this strain on the various media was no greater than the difference between the growths of the different strains from dasheens, or between the growths in different tubes of the

same strain. The one striking exception to the above statement may be noted in connection with the results obtained with saccharose, lactose, dextrose, and glycerin broth in fermentation tubes. Strain 3624 produced gas (a small amount) in all, while none of the other strains did. Such a difference, however, is not surprising in view of the fact that Harding and Morse (4) found that of the various strains from different sources studied by them some consistently failed to produce gas.

The writer wishes to emphasize in this connection that he has carefully compared his results with the studies of Jones (5) and Harding and Morse (4) and has frequently consulted Smith's "Bacteria in Relation to Plant Diseases" (8) for methods. Slight differences in cultural characteristics have been noted from time to time between the different strains, but these differences appear to be no greater than would naturally be expected between strains of the same organism. No attempt has been made to duplicate all the work of Jones or of Harding and Morse with this group of organisms, but merely to carry the work of comparison far enough to be reasonably sure that the writer was working with a strain similar to or identical with *B. carotovorus*.

By a series of cross-inoculations it was shown that the organism furnished by Dr. Jones would decay dasheens and the organisms from dasheens softrotted raw carrots and turnips. It should be emphasized in this connection that strain 3624 (Jones) was less virulent for dasheens than the strain isolated from dasheen, though it rotted carrots and turnips with ease.

## INOCULATION EXPERIMENTS

As a preliminary test, 12 sterile raw blocks in test tubes with a little water added were cut from corms and inoculated on April 1, 1915, with a 24-hour-old culture (organism 3595) on beef bouillon. In three days there was evidence of decay in some of the tubes, and in 10 days four of the blocks were completely rotted. The checks, six in number, remained sound. The causal organism was recovered in pure culture from two of the blocks.

On April 19, 1915, twelve more sterile raw blocks and also six dasheen tubers in moist chambers were inoculated with a 3-day-old culture of beef bouillon by placing a loopful of the broth in a depression of a cut surface. The raw blocks in test tubes were all decayed by April 24. Four of the tubers in moist chambers were well rotted on the same date and the other two but slightly. The causal organism was reisolated from four. Six raw blocks in tubes and three tubers in moist chambers were held as checks. All remained sound. The lack of material prevented further work at this time. The work was again taken up in November, the tubers or corms being cut in two and inoculation made on the wounded surface in moist chambers, the surface being kept moist for

a day or two by spraying with sterile water from an atomizer. Cultures from beef bouillon were used for all inoculations.

On November 27 eight tubers were inoculated with organism 3616 from a 3-day-old culture and all were completely decayed in seven days. The organism was recovered from four. Strain 3616 was isolated from a Pat-long-fu taro (*Colocasia esculenta*) on November 18, 1915. The corm was decayed at the base and was lifted a few days before in the condition shown by Plate LXXXIII. Ten raw sterile blocks inoculated on the same day with each of strains 3595 and 3616 were completely decayed in three days. On November 24 ten tubers were inoculated with organism 3624 (*Bacillus carotovorus* from Dr. L. R. Jones) from a 24-hour-old culture. A slight rot had taken place in seven days; and in 12 days, although the decay had increased, it was still slight. The causal organism was recovered from three tubers. On December 1 eight tubers were inoculated with strain 3624, and in five days about half of each tuber was decayed. The organism was recovered from four. The checks, six in number, remained sound. On December 9 six raw carrot and six raw turnip blocks in test tubes were inoculated with strains 3616 and 3595. Decay started in 24 hours and was complete in five days. At the same time thirteen raw blocks of dasheens were inoculated with strain 3624 from a 2-day-old culture, and in 5 days nine blocks were completely decayed; the others and the ten checks remained sound. On December 13 three turnips and three carrots in moist chambers were inoculated with a 6-day-old culture of strain 3595, and by December 20 two turnips and one carrot were completely decayed. At the same time six dasheen tubers were inoculated with a 6-day-old culture of strain 3624. Decay began in 24 hours and in seven days had destroyed most of each tuber. The four checks remained sound. On December 20, 1915, four turnips and four carrots were inoculated with a 7-day-old culture of organism 3627 (a reisolation of 3616) and three turnips and four carrots with strain 3595. A rot started in 48 hours and decay was complete in seven days. The causal organism was recovered from the four turnips inoculated with strain 3627 and from the four carrots inoculated with strain 3595. The ten checks remained sound.

On December 27 four dasheens were inoculated with 1-day-old cultures of strain 3616 and six carrots and seven turnips with strain 3624. By January 3 the dasheens were nearly decayed and all the turnips and five of the carrots completely rotted. All the checks remained sound. Eleven turnips inoculated with 1-day-old cultures of strain 3616 were completely decayed by January 3, 1916. Four dasheen corms inoculated with 1-day-old culture of 3624 were but slightly rotted at the end of seven days. On December 28 three turnips and four carrots were inoculated with a 24-hour-old culture of organism 3626 (an isolation from a fibrovascular bundle at the center of a large corm). In six days

all but one turnip was nearly rotted. The four checks remained sound. On December 31 five turnips and five carrots were inoculated with a 48-hour-old culture of organism 3627. By January 9, 1916, three turnips and four carrots were badly rotted. The organism was recovered from all the decayed specimens. On January 6, 1916, four dasheen corms were inoculated with a 3-day-old culture of strain 3624. By January 13 a slight rot had taken place. The rot progressed but little in one more week and the specimens were thrown out. On January 7 six turnips and six carrots were inoculated with a 1-day-old culture of strain 3627 and by January 12 all were completely decayed. The causal organism was received from six. The seven checks remained sound.

Table IV gives the results of the inoculation experiments with *Bacillus carotovorus*.

TABLE IV.—*Results of inoculation experiments with Bacillus carotovorus*

| Strain No. | Date of inoculation. | Host. | | | Inoculated. | Infected. | Checks. | Checks infected. | Reisolations. |
|---|---|---|---|---|---|---|---|---|---|
| | | Dasheen. | Turnip. | Carrot. | | | | | |
| | 1915. | | | | | | | | |
| 3595 | Apr. 1 | Raw blocks | | | 12 | 4 | 6 | 0 | 2 |
| 3595 | Apr. 19 | do | | | 12 | 12 | 6 | 0 | 0 |
| 3595 | do | Tubers | | | 6 | 4 | 3 | 0 | 0 |
| 3595 | Nov. 27 | Raw blocks | | | 10 | 10 | 0 | 0 | 4 |
| 3595 | Dec. 9 | | Raw blocks | | 6 | 6 | 10 | 0 | 0 |
| 3595 | Dec. 13 | | Roots | | 3 | 2 | 0 | 0 | 0 |
| 3595 | Dec. 20 | | do | | 3 | 3 | 10 | 0 | 0 |
| 3595 | Dec. 9 | | | Raw blocks | 6 | 6 | 10 | 0 | 0 |
| 3595 | Dec. 13 | | | Roots | 3 | 1 | 0 | 0 | 0 |
| 3595 | Dec. 20 | | | do | 4 | 4 | 10 | 0 | 4 |
| 3616 | Nov. 27 | Tubers | | | 8 | 8 | 0 | 0 | 4 |
| 3616 | do | Raw blocks | | | 10 | 10 | 0 | 0 | 0 |
| 3616 | Dec. 27 | Tubers | | | 4 | 4 | 2 | 0 | 0 |
| 3616 | Dec. 9 | | Raw blocks | | 6 | 6 | 10 | 0 | 0 |
| 3616 | Dec. 27 | | Roots | | 11 | 11 | 10 | 0 | 0 |
| 3616 | Dec. 9 | | | Raw blocks | 6 | 6 | 10 | 0 | 0 |
| 3624 | Nov. 27 | Tubers | | | 10 | [a]10 | 0 | 0 | 3 |
| 3624 | Dec. 1 | do | | | 8 | 8 | 6 | 0 | 4 |
| 3624 | Dec. 9 | Raw blocks | | | 13 | 9 | 10 | 0 | 0 |
| 3624 | Dec. 13 | Tubers | | | 6 | 6 | 4 | 0 | 0 |
| 3624 | Dec. 27 | | Roots | | 6 | 6 | 10 | 0 | 0 |
| 3624 | do | | | Roots | 7 | 5 | 10 | 0 | 0 |
| 3626 | Dec. 28 | | Roots | | 3 | 2 | 4 | 0 | 0 |
| 3626 | do | | | Roots | 4 | 4 | 4 | 0 | 0 |
| 3627 | Dec. 20 | | Roots | | 4 | 4 | 10 | 0 | 4 |
| 3627 | Dec. 31 | | do | | 5 | 3 | 0 | .... | 3 |
| 3627 | Dec. 20 | | | Roots | 4 | 4 | 10 | 0 | 4 |
| 3627 | Dec. 31 | | | do | 5 | 4 | 0 | .... | 4 |
| | 1916. | | | | | | | | |
| 3624 | Jan. 6 | Tubers | | | 4 | [a]4 | 0 | .... | 0 |
| 3627 | Jan. 7 | | | Roots | 6 | 6 | 3 | 0 | 3 |
| 3627 | do | | Roots | | 6 | 6 | 4 | 0 | 3 |

[a] All slightly.

## OTHER FUNGI ISOLATED AND STUDIED

In storing on the ground a crop such as dasheens it is only natural that a number of saprophytes would be associated with the storage-rot organism. Two species of Fusarium, *F. oxysporum* and *F. caudatum*, were frequently isolated under such conditions. Although preliminary inoculation experiments made by inserting spores and hyphæ of these organisms with a needle or by smearing spores on a cut surface of the tubers in a moist chamber gave negative results, it was still believed that they would produce true storage-rots under the proper conditions. As the writer believed that sufficient moisture was lacking, the tubers, after being dipped in spore suspension in sterile water, were wrapped with wet filter paper and then with oiled paper and placed in a moist chamber. The results were negative. Tubers soaked for one hour in water and then dipped in a spore suspension and wrapped in filter paper and oiled paper remained sound. Again, tubers kept at á temperature of about 12° C. for 10 days, inoculated and manipulated as above, and kept in a moist chamber yielded no result. It was finally concluded from these results, in view of the fact that other organisms readily cause storage-rots under laboratory conditions, that these two fungi were merely saprophytes. A species of Phomopsis isolated from dasheens from the Hawaiian Islands failed to produce a rot under any of the conditions tried. Other fungi isolated a few times but not studied were *Rhizopus nigricans*, *Penicillium* spp., *Pythium debaryanum*, *Fusarium redolens*, and an undetermined species of Fusarium.

A number of inoculations were made with *Diplodia zeae*, *Sphaeropsis malorum*, and a species of Diplodia from salix, none of which produced a rot.

## MOISTURE AS A FACTOR IN PRODUCING ROT

It is likely that moisture plays a far greater part in the production of storage-rots than is generally conceded. Ordinarily it might be supposed that the amount of humidity in a moist chamber lined with saturated filter paper would be sufficient to germinate the spores of most fungi. *Fusarium solani* under those conditions would not invade the tissue of dasheens; but if they were sprayed twice a day for one or two days so that the spores would be suspended in a film of water germination and invasion of the tissue would take place before an impenetrable corky layer had formed over the wound. Some root crops have the power to absorb a considerable quantity of water, so that even though water of condensation may be formed on the glass of a moist chamber, the specimen inside is comparatively dry. For example, five tubers of dasheens with a total weight of 558 gm. absorbed 21 gm. of water in 24 hours, or more than 3.7 per cent of their original weight; and ten sweet potatoes from storage with a total weight of 1,539 gm. absorbed 84 gm. in two hours, or nearly 5.5 per cent of their original weight. Both

dasheens and sweet potatoes continue to absorb water for some time, and sweet potatoes will take up as much as 7 to 20 per cent of their weight in 24 hours, depending naturally on how dry they were when immersed. Relatively the greatest absorption takes place during the first two hours; in extreme cases as much as 10 per cent. The rate of absorption drops off at the end of that time, but the curve continues steadily upward thereafter.

*Sclerotium rolfsii* also requires considerable moisture to start growth, but requires no addition of water to that in the filter paper of a moist chamber after 24 hours. This fungus may have the power after once becoming well established to penetrate the corky layer over a cut surface. Whether this is accomplished by the action of an enzym was not determined.

*Diplodia tubericola* and the other closely related forms used in these experiments succeed better under exactly the opposite conditions. If the tubers after inoculation were subjected to the environment of the laboratory room, the results were better than if they were kept in a moist chamber. No attempt has been made to determine a cause for this phenomenon. It must be kept in mind that at the outset protection was afforded the spores and hyphæ by inserting them about a fourth of an inch into the tuber and the tissue squeezed together about the wound.

*Bacillus carotovorus*, like *S. rolfsii* and *F. solani* succeeded better if a film of moisture was sprayed on the cut surface for a day or two following inoculation. As soon, however, as decay set in, no further application was required, except to the filter paper in the bottom of the moist chamber. It should be noted in this connection also that dasheens, turnips, and carrots differ very much in respect to the moisture actually required to stimulate decay. Dasheens are very dry and absorb moisture quickly and must be sprayed several times to start decay. Turnips and carrots, on the other hand, require but little added moisture, decay starting more promptly and progressing more rapidly.

## TEMPERATURE AS A FACTOR IN PRODUCING ROT

Temperature and moisture, so far as their relation to storage rots are concerned, are so closely associated that one can hardly be discussed independently of the other.

It is obvious that decay will not occur at a temperature at which the organism will not grow even in the presence of sufficient moisture or in the absence of moisture with the proper temperature.

RESULTS WITH DIPLODIA TUBERICOLA.—A number of dasheens inoculated with *Diplodia tubericola* from sweet potatoes were divided into two lots, one of which was placed in an incubator with a temperature varying from 34° to 35° C. The other lot was placed in an ice box with a temperature ranging from 12.2° to 13.5°. At the higher temperature

(34° to 35°) the tubers were more than half-rotted at the end of nine days, and in four more days all but one were decayed throughout. At the end of 20 days *D. tubericola* was isolated in pure culture from each. At a lower temperature (12.2° to 13.5°) all the tubers but one were perfectly sound at the end of 45 days. One tuber was half-decayed, and yielded *F. culmorum*.

The temperatures at which decay may be brought about by *S. rolfsii*, *F. solani*, and *B. carotovorus* were determined by the use of raw blocks of dasheen. After discarding the blocks contaminated in their preparation, the remainder were divided into two lots, one of which was inoculated with *S. rolfsii* and the other with *F. solani*. Each of these lots was divided into 6 groups of 10 tubers each and placed in different chambers of the Altman thermostat and in the laboratory room, the temperatures of which ranged as follows:

| Chamber No. | Range of temperature. | Average temperature. | Chamber No. | Range of temperature. | Average temperature. |
|---|---|---|---|---|---|
| | °C. | °C. | | °C. | °C. |
| 5 | 8.2 to 10.0 | 9.1 | Room | 22.0 to 24.0 | 22.4 |
| 6 | 12.0 to 15.0 | 14.0 | 18 | 26.3 to 29.6 | 28.6 |
| 9 | 17.5 to 19.5 | 18.4 | 19 | 34.5 to 36.0 | 35.3 |

RESULTS WITH FUSARIUM SOLANI.—In all the chambers except No. 5 (9.1° C.) growth started in two days. While there was some difference in the general appearance of the growth in the different chambers, there was nothing strikingly characteristic. At the lower temperatures there was a slight reduction of hyphal growth compared with higher temperatures, accompanied by the production of a salmon-orange color on the blocks. At the higher temperatures, particularly in chambers 18 (28.6°) and 19 (35.3°), abundant hyphæ were produced. An accident to chambers 18 and 19 at the end of 10 days terminated that part of the experiment, but an examination of the tubes showed that the blocks were completely decayed and typical spores of the causal organism produced. The others were continued for 20 days longer. At the end of that time no decay had taken place in chamber 5 (9.1°), and no spores were formed, though a slight discoloration of the blocks had taken place. In all the other chambers the blocks were completely softened. In the tubes exposed to room temperature (22.4°) typical spores were produced, while in chamber 6 (14.0°) there were a few abnormal spores, in No. 9 (18.4°) many. In general it may be stated that while decay was complete in all chambers except in No. 5 (9.1°) spore production was better at the three higher temperatures. The results therefore seem to indicate that tubers stored at the higher temperatures are more liable to be decayed by *F. solani* than if stored at a temperature of 8° to 10° or lower.

RESULTS WITH SCLEROTIUM ROLFSII.—This organism produced no decay at the end of 38 days in chamber 5 (9.1° C.), but a few immature sclerotia were formed. In all the other chambers visible growth appeared in two days. In chambers 6 (14.0°) and 9 (18.4°) hyphæ were abundantly produced and the sterile blocks completely decayed, but no sclerotia were produced at the conclusion of the experiments. At the three higher temperatures the blocks were also decayed, but the production of hyphæ was markedly less and the number of sclerotia relatively larger, increasing in number with the increase in temperature. It therefore appears that the minimum temperature at which this organism will produce decay is near 8° to 10°. Other things being eliminated, dasheens would apparently, from the results of these experiments, keep better if stored at a temperature of about 8° to 10°.

RESULTS WITH BACILLUS CAROTOVORUS.—Experiments to determine the range of temperature of *B. carotovorus* were made some months later by inoculating sterile raw blocks of turnips and dasheens. The blocks were inoculated with a 24-hour-old culture of strain 3616 grown in beef bouillon and exposed in a series of chambers of the Altman thermostat and in the laboratory room to the following average temperatures:

| Chamber No. | Range of temperature. | Average temperature. | Chamber No. | Range of temperature. | Average temperature. |
|---|---|---|---|---|---|
| | °C. | °C. | | °C. | °C. |
| 2 | 3.5 to 5.0 | 4.0 | 9 | 15.7 to 20.0 | 17.3 |
| 3 | 5.2 to 7.0 | 6.1 | Room | 20.0 to 25.0 | 23.0 |
| 5 | 9.0 to 12.0 | 10.0 | 18 | 29.2 to 36.2 | 32.7 |
| 6 | 11.0 to 14.8 | 12.3 | 19 | 34.7 to 37.9 | 35.8 |
| 8 | 14.6 to 18.5 | 16.0 | 20 | 38.0 to 41.6 | 39.3 |

The tubes were kept in the chambers for 27 days. At the end of that time no growth had taken place in chamber 2 (4° C.) and but a slight growth in 3 (6.1°), and 20 (39.3°). In six days a slight decay had started in chamber 5 (10°) and in three days in chamber 6 (12.3°). At the end of 14 days the dasheens were completely decayed in chamber 5. The turnips, on the other hand, were only partially decayed at the close of the experiment. In chamber 6 the dasheens were completely decayed at the end of 11 days and the turnips nearly so at the end of 14 days. In all the other chambers decay was noticeable at the end of two days, but progressed more rapidly with the increase of temperature up to and including 18 (32.7°). At the end of 11 days the dasheens were completely decayed in chamber 8 (16°) and the turnips mostly so, while in 9 (17.3°) the dasheens were completely decayed in 7 days and the turnips in 11 days. In chamber 18 (32.7°) both dasheens and turnips were completely decayed in 3 days, while in 19 (35.8) decay was not complete until 10 days. A parallel series of tests was run

in the laboratory room (23°), and decay was completed in 10 days. From these results there is a wide range of temperatures at which decay by this organism will take place. It is apparent, however, that the optimum lies somewhere between 32° to 35° and the minimum at approximately 4°. The maximum temperature was not determined, but in view of the fact that a slight decay of the blocks occurred in chamber 20 (39.3°), it must be somewhat higher.

It is interesting to note in this connection that the dasheens in most of the chambers were more promptly decayed in this experiment than the turnips. In other experiments of a similar nature both in the laboratory room and in the thermostat chambers this has not always been the case. In fact, it has frequently happened that the turnips, and carrots also when they are included in the tests, were more promptly decayed than the dasheens. While a strain (3616) originally obtained from dasheens was used for inoculating the blocks, turnips and carrots inoculated with this strain in moist chambers were generally more speedily decayed than dasheens. While no positive explanation of such a condition will be attempted, it has been apparent throughout the whole course of the work that the condition of the material when used plays no little part in the results to be obtained. It has been noticed that fresh turnips and carrots decay after inoculation more readily than those that have been kept in the ice box or elsewhere under conditions permitting the escape of moisture and eventual withering. Dasheens, on the other hand, lose moisture more slowly and remain suitable for such experiments a much longer time.

## SUMMARY

(1) There are four storage rots of economic aroids: Java blackrot caused by *Diplodia tubericola*, *Diplodia maclurae*, *Diplodia gossypina*, and *Diplodia* sp. from *Mangifera indica;* powdery grayrot caused by *Fusarium solani;* sclerotium-rot caused by *Sclerotium rolfsii;* and softrot caused by *Bacillus carotovorus*.

(2) All of the species of Diplodia cause a rot identical in character.

(3) All the causal organisms are wound parasites.

(4) The parasitism of each organism has been established by inoculation experiments.

(5) *F. solani* from the Irish potato produces a rot identical with the rot produced by *F. solani* from the dasheen.

(6) Several other organisms were studied, none of which were found parasitic.

(7) The Java blackrot organism produced decay better under relatively dry conditions.

(8) It was necessary to apply sterile water once or twice to the tubers and corms after inoculation with *F. solani*, *S. rolfsii*, and *B. carotovorus*. After decay had started, no further application of water was required.

(9) High temperatures were more favorable to decay than low temperatures.

(10) *B. carotovorus* alone produced decay at an average temperature below 9° C.

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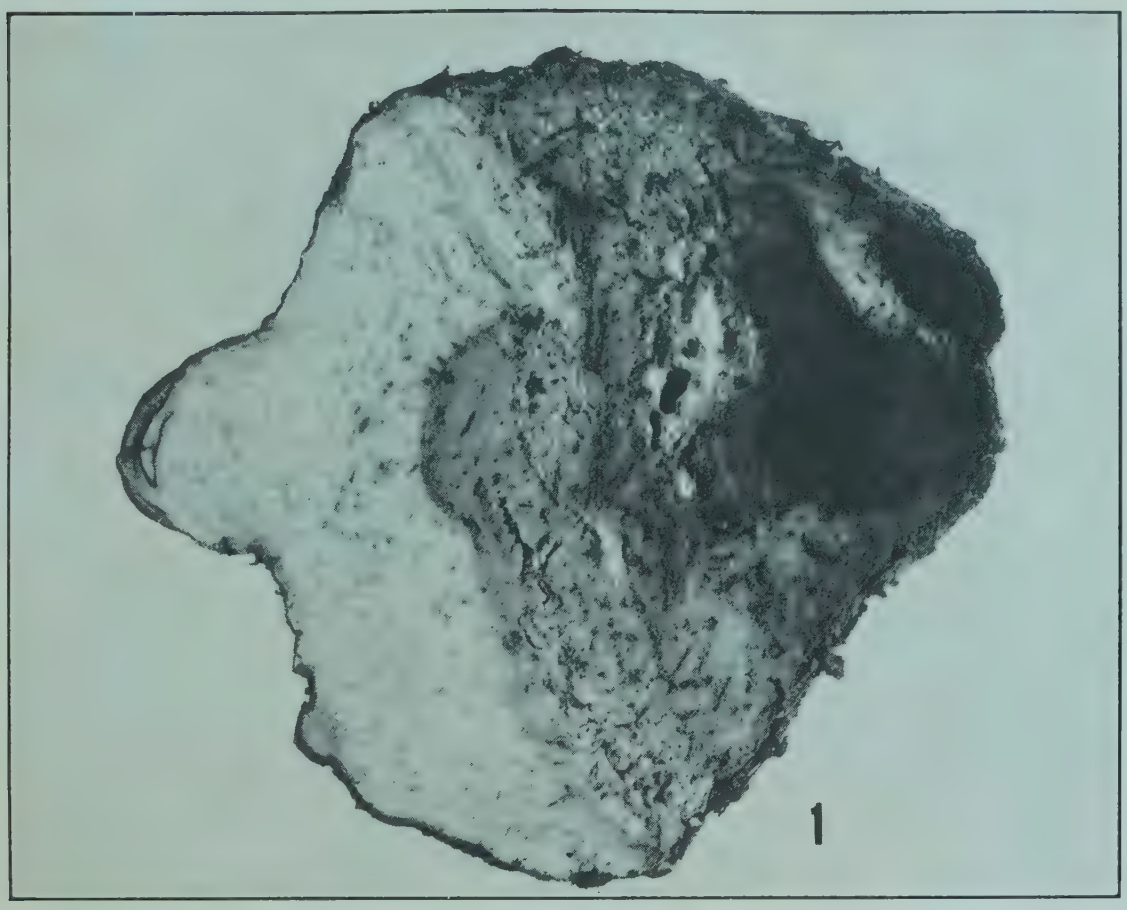
## PLATE LXXXI

Fig. 1.—A dasheen corm (*Colocasia esculenta*) showing Java blackrot produced by *Diplodia tubericola*. The blackrot end of the corm is separated from the healthy tissue by a dark brown area which in turn blackens later. Field material from Brooksville, Fla.

Fig. 2.—A corm of *Alocasia sp.* showing Java blackrot produced by *D. tubericola*. From a laboratory inoculation.

Fig. 3.—A dasheen tuber partially decayed by *Sclerotium rolfsii*. From a laboratory inoculation.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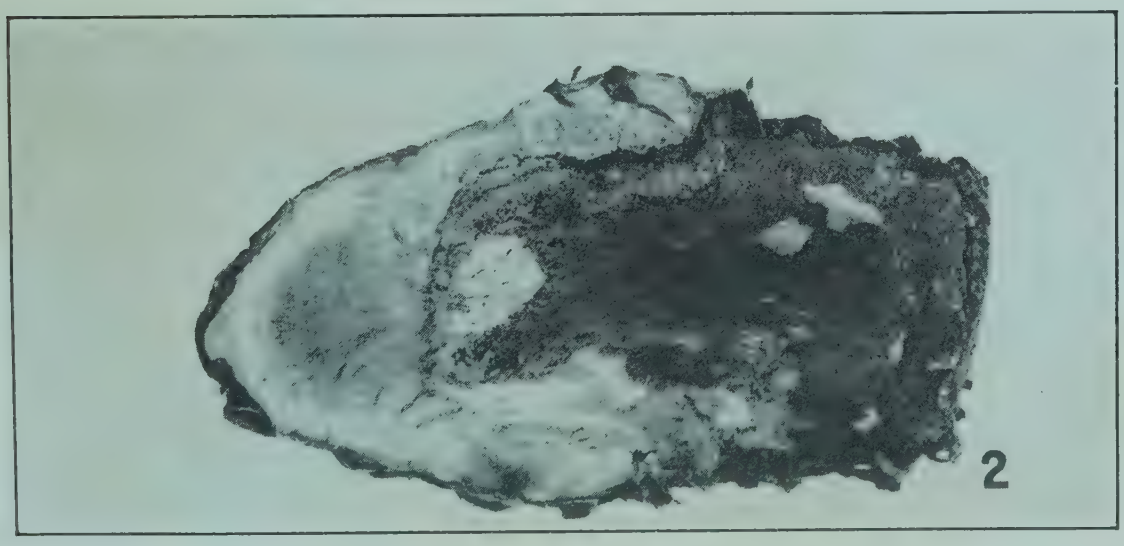
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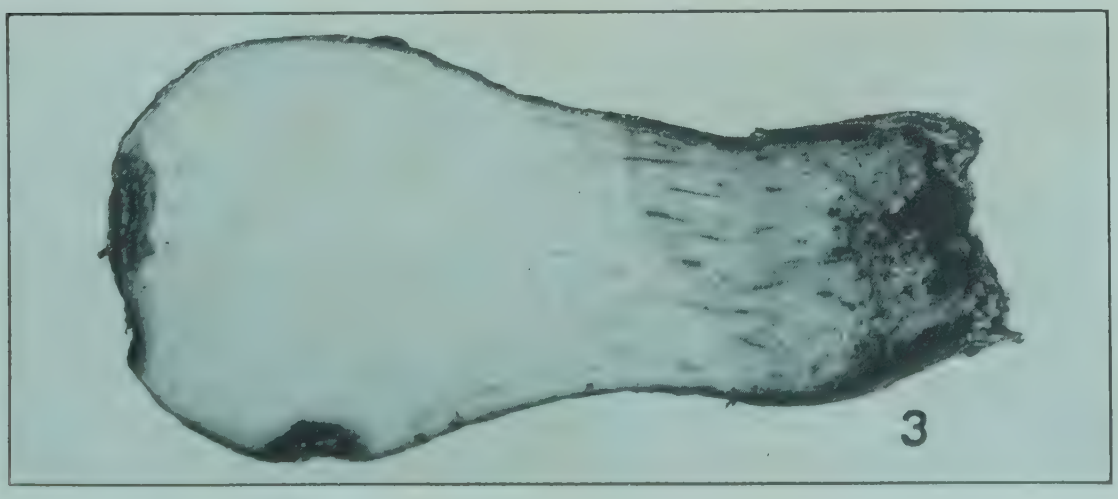
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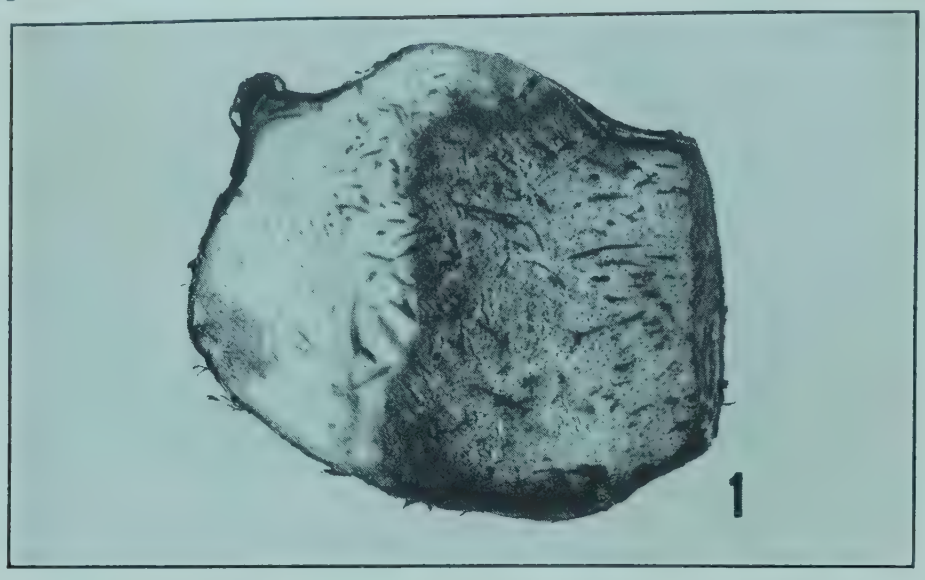
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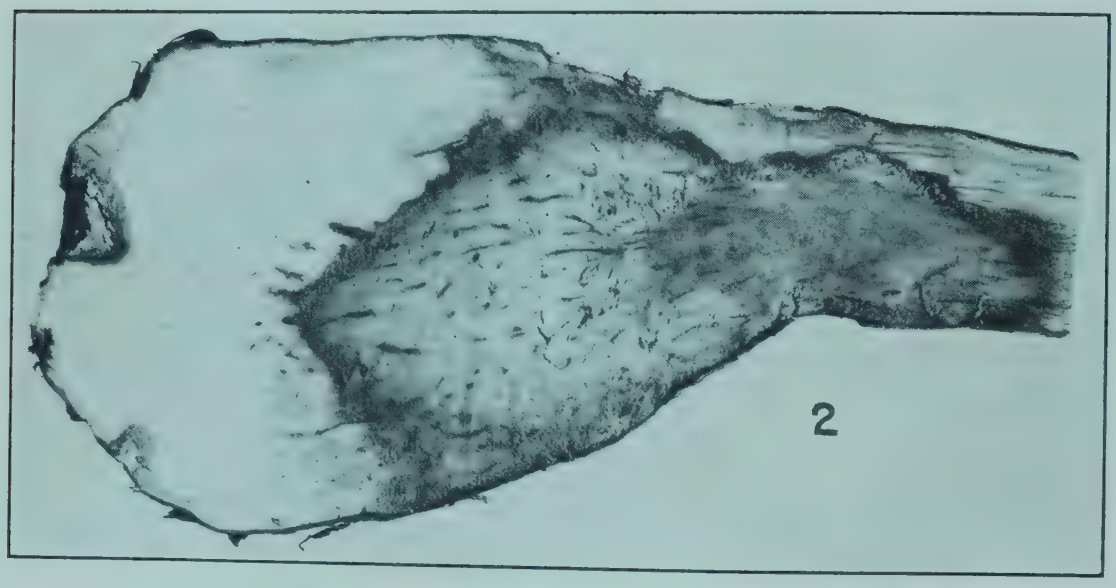
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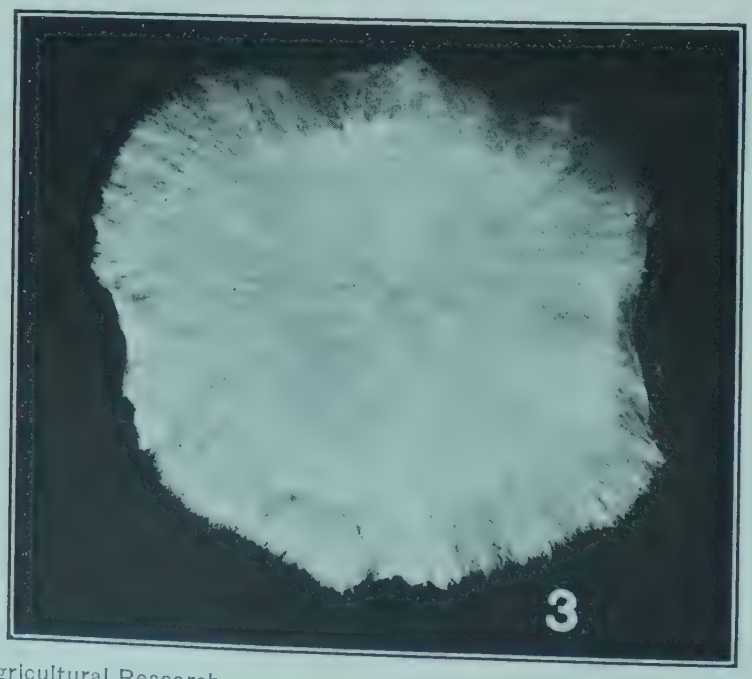
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## PLATE LXXXII

Fig. 1.—A tuber of *Colocasia esculenta* showing a powdery grayrot caused by *Fusarium solani*. From a laboratory inoculation.

Fig. 2.—A tuber of *Xanthosoma sagittifolium* showing partial decay by *Fusarium solani*. From a laboratory inoculation.

Fig. 3.—A tuber of *C. esculenta* softened throughout by *Sclerotium rolfsii*. Note the hyphae over the entire surface. From a laboratory inoculation.

## PLATE LXXXIII

A corm of *Colocasia esculenta* from Brooksville, Fla., mostly rotted away by *Bacillus carotovorus*. The organism isolated from this corm produced positive laboratory infections.

# EXPERIMENTS WITH CLEAN SEED POTATOES ON NEW LAND IN SOUTHERN IDAHO

[PRELIMINARY PAPER]

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It has generally been assumed by plant pathologists that if disease-free potatoes (*Solanum tuberosum*) were planted on new land the resulting product would be free from disease. For the past three years the writer has been engaged in investigations of potato diseases in southern Idaho, where this crop is grown under irrigation. As these irrigated tracts have but recently been opened up, there are many acres of land which may be classed as new in every sense of the word, since no agricultural crops have ever been grown upon them. Pathologists and potato growers alike believed that in these new lands just reclaimed from the desert lay a wonderful opportunity for the production of disease-free potatoes. However, from the beginning of the potato-growing industry in the irrigated portion of southern Idaho potato diseases have appeared each year. It is known that the first seed planted by the potato growers of these irrigated tracts was far from being free from disease, and it was naturally assumed that the diseases which appeared in the product had been introduced with the seed planted. The diseases most prevalent are wilt (*Fusarium oxysporum* Schlect.), blackrot (*F. radicicola* Wollenw.), jelly-end rot (*Fusarium* sp.), Rhizoctonia or russet scab, powdery dryrot (*F. trichothecioides* Wollenw.), and common scab.[1]

During the first two years of the author's investigations of potato diseases in southern Idaho, he observed that when potatoes were planted on virgin land just reclaimed from the desert many diseases usually appeared. Often the product from potatoes planted on such land appeared to be more diseased than that from potatoes planted on land which had been reclaimed from the desert for several years and which had been planted with other crops, such as alfalfa or grain. Frequently when such a diseased crop was observed, the grower would insist that the seed potatoes he had planted had been practically free from disease. Since certain of the diseases found, such as common scab and blackrot, are easily detected on the seed, the writer was forced to admit that in many such cases the grower might be right. Therefore,

[1] No attempt has been made to isolate an organism from the common scab found in this region, but since its appearance is identical with that found in the East it is assumed that the causal organism is the same—namely, *Actinomyces chromogenus* Gasperini.

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in the spring of 1915, experiments were set up to determine whether a clean product could be obtained by planting disease-free seed on new land. While these experiments are to be continued another year, the results of the first year's trials were so conclusive and of such importance to the potato-growing industry that it appears desirable to record them at the present time.

In the spring of 1915 arrangements were made with several farmers to plant clean seed on lands which had never before been planted to potatoes. The plots planted ranged from one-twentieth of an acre to 1 acre in size. Six of the plots were planted on virgin soil reclaimed from the desert for the express purpose of planting with disease-free seed potatoes. Fourteen of the plots were planted on land which had for several years been in alfalfa or grain. On the grounds of the experiment station at Jerome, Idaho, other plots were planted with disease-free seed.

The land at the experiment station was reclaimed from the desert in 1910, planted to barley, and thereafter to alfalfa.

The varieties planted in the test plots were as follows: Idaho Rural, Netted Gem, Rural New Yorker, Pearl, Peoples, Red Peachblow, Burbank, Carmen No. 3, and Early Six Weeks. The disease-free seed was selected in the same manner for each plot as follows: Each tuber was first carefully examined for all external evidence of disease, such as common scab and the sclerotia of *Rhizoctonia* sp. All tubers showing evidence of either of these diseases were rejected. No tubers showing any large amount of infection with powdery dryrot were used. If there was only a small pocket of dryrot present, the infected portion was cut out until the tissues appeared white and clean. The externally clean tubers were then cut, the first cut being made across the stem end. The stem end portion was invariably discarded. If there was no evidence of vascular or other discoloration, the balance of the tuber was considered free from disease and was cut into pieces averaging about 2 ounces each. After cutting, the tubers were disinfected for 1½ hours in a solution of mercury bichlorid (1:1,000).

Throughout the season each plot was carefully watched, cultures being made from time to time as evidence of disease appeared in the plants. Wilt was found in every plot and *Fusarium oxysporum* was obtained in artificial cultures from stems showing vascular discoloration. Stem lesions and footrots were especially severe in all of the desert (or virgin) land plots. In all of the desert-land plots the plants presented a sickly appearance as compared with the plants in the alfalfa and grain land plots. There were indications in each of the desert-land plots of light yields and of a diseased product.

At harvest time the following methods were employed to determine the diseased condition of the tubers: In each of the smaller plots 100 hills were dug and the product of each hill examined separately. The

tubers were first examined for the presence of external diseases, such as Rhizoctonia or russet scab, common scab, blackrot, and jelly-end rot, after which each tuber was cut to determine the presence or absence of infection in the vascular tissue. The method employed in each of the larger plots was the same as in the smaller ones, except that several lots of 100 hills each were dug in different parts of each plot. All tubers showing pronounced vascular discoloration were considered as infected with wilt caused by *Fusarium* spp. Tubers showing such discoloration were taken to the experiment station laboratory and cultures were made from the discolored vascular tissue. Eighty per cent of all such cultures showed the presence of either *F. oxysporum* or *F. radicicola*. The percentage of vascular infection present in the harvested product was estimated on this basis.

The average percentage of disease present in the alfalfa-grain land plots, planted with disease-free seed, including the plots at the Jerome experiment station, was as follows: Common scab, 4.7 per cent; Rhizoctonia or russet scab, less than 2.8 per cent; vascular infection, 26 per cent; and fieldrots caused by *Fusarium* spp., less than ½ of 1 per cent. In the desert-land plots the averages were as follows: Common scab, 9.3 per cent; Rhizoctonia or russet scab, 11.6 per cent; vascular infection, 29.3 per cent; and fieldrots caused by *Fusarium* spp., 5.6 per cent. The fieldrots caused by species of Fusarium are blackrot (*F. radicicola*) and jelly-end rot, the causal organism of which has not been definitely determined, but with it are associated *F. radicicola* and *F. oxysporum*, as well as other species of Fusarium. Of these two fieldrots, blackrot was the one principally found. Jelly-end rot was confined to the Netted Gems and rarely occurred.

It will be seen that the percentage of disease was much higher in the plots planted on virgin soil than in the plots planted on land which had previously been cropped with alfalfa or grain. When the fact is taken into consideration that the yield in each of the desert-land plots was light and the tubers small and of poor quality, it must be admitted that raw desert lands are not well adapted to the production of high-grade seed stock.

From the results so far obtained from the experiments the following conclusions are drawn:

(1) Planting clean seed potatoes on new land does not guarantee a disease-free product.

(2) A smaller percentage of disease may appear in the product when clean seed is planted on alfalfa or grain land than when similar seed is planted on virgin or raw desert land.

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# DIGESTIBILITY OF VERY YOUNG VEAL

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## INTRODUCTION

Throughout the United States little was known until recently regarding very young veal, since the sale of calves less than 3 to 6 weeks old for food is prohibited by Federal and State laws. Our attitude toward veal, as toward many other foods, has been determined in part by custom and prejudice and in part by economic conditions and experience, often being illogical; therefore it is of interest to ascertain in such cases how far belief is justified by facts, as shown by controlled experimental tests.

That the common opinion that veal is less wholesome than beef and young veal less so than mature veal is not a consistent prejudice against young flesh foods is shown by the common and apparently growing taste which prefers squab to pigeon, ranks broilers as superior to fowls, considers sucking pig a great delicacy, and regards hothouse lamb—that is, lamb less than 3 months old and rapidly grown and fattened—as much superior to older lamb as lamb is to mutton.

That economic conditions may have an effect upon opinion, which is not consistent, is shown by the situation with respect to the marketing of calves. In grazing areas where the cheapness of food makes it possible to rear cattle at least to early maturity there is a natural tendency to do so. In regions where the dairy industry is highly developed, milk is such an important product that it is not thought profitable to rear calves beyond the period when the mother's milk becomes salable, and so, even though they can not be marketed and the producer will seldom care to use on his own table what he is prohibited from selling, they are often slaughtered at from 3 to 6 days old instead of fed until the lawful marketable age.

The prejudice against veal, and more particularly young veal, is inspired chiefly, it would seem, by the belief that it is indigestible, by which is meant either that it causes a digestive disturbance or that it

fails to digest as thoroughly as other meats, with the result that it is either harmful or undesirable as food. That this opinion does not rest on general experience is indicated by the contrary belief in Europe, which ranks veal as a particularly desirable meat, even for invalids, and which regards very young veal much as it does young pig and young lamb.

The question of its dietary value and its digestibility, both in the more popular as well as in the technical sense, thus becomes one worthy of study for itself and for its bearing upon the common prejudice against the use of young veal as well as upon the related matter of wholesomeness when this food is eaten in comparison with more commonly accepted foods, and accordingly the tests here reported were undertaken.

## PREVIOUS EXPERIMENTS

In the literature consulted empirical conclusions are not uniform and very little definite information has been found regarding the food value and possible use in the diet of very young veal.

Studies have been made, however, to determine what the difference is, chemically or otherwise, between very young veal and the older market veal. Fish (4),[1] for instance, conducted such an investigation, with the object of obtaining data which would enable him to determine the relative age of the animal in market, so that the very young could be detected. He determined the specific gravity and freezing point of the tissue juice and also the percentage of water, finding that in very young veal, where more water is present in the tissues, there is less depression of the freezing point and a lower specific gravity. In continuation of this work, the same author (5) made dietary studies to determine whether the flesh of the young calf from 1 to 14 days of age exerts any injurious effects upon the consumer. Seven families, including over 20 individuals from 2 to 60 years of age, ate this meat and reported no physiological disturbances, the health of each remaining apparently normal. Later, the results of experiments in vitro led Fish (6) to conclude that the difference in the thoroughness of digestibility of the tissues of very young and market veal is so small as to be practically negligible.

Very recently Berg (3) has reported the experimental data of a biochemical comparison of beef and immature veal in which he discusses the chemical composition, digestion in vitro, and results of feeding experiments made with animals (cats) in the laboratory. He concludes that there are no physiologically significant differences in the chemical composition of beef and immature veal. In a large number of artificial digestion experiments he found that immature veal was as quickly digested as beef. In the feeding experiments with cats, immature veal supplied all of the nitrogen and a large share of the energy of the

[1] Reference is made by number to "Literature cited," p. 587–588.

diet. The animals remained in a normal condition at all times, exhibiting characteristic functions of growth, maintenance, and reproduction. Berg reports that the immature veal at times was kept in an ice box at 2° to 4° C. for many days before use and that it remained in edible condition.

It is evidently the opinion of the more recent investigators that very young veal, or, as it is commonly called, bob veal, is not unsuited for use as human food. As very little information, however, is available as regards the coefficient of digestibility of very young veal, a series of experiments was undertaken to determine the completness of digestion of this material by the human subject in normal health.

## DIETARY TESTS

Before attempting to study the digestibility of very young veal, tests were made in which it was cooked in the laboratory and in several homes and eaten in quantity, although no record was kept of the amounts of veal and other foods eaten. The purpose of these tests was to have very young veal prepared by different methods and eaten by a large number of persons whose ages and daily activities were quite varied, to see whether purging or other disturbances of digestion would result and whether there was warrant for the popular belief that it is indigestible in the sense that it causes illness or distress. In general, it may be said that no physiological disturbances were noted either in the laboratory tests with individuals or in the tests made in a number of families.

Reports of the individual tests of the use of very young veal are as follows:

In family A the ages of the various members ranged from 4 to 65 years. Observations of the dietary value of bob veal were made at various times, using different portions of the carcass. With one exception no member of the family was apprised of the age of the veal, which was cut up into small pieces and prepared in the form of a stew. The criticism offered in regard to the meat was that it seemed somewhat dry, and one member of the family remarked that it seemed to be stringy. No one experienced any ill effects whatever from eating the meat, and all appeared to relish it as much as market veal.

Family B was comprised of comparatively young adults only. All the members of the family were apprised of the nature of the meat, which was served in the form of a roast. Their criticism of the veal was that it seemed rather tasteless—that is, lacked flavor—and that although it appeared good it would not be preferred to the ordinary market veal. No instances of any ill effects resulted from eating this meat.

Family C was composed of both children and adults whose ages ranged over a period of 50 or more years. In this study tests were made at two different times. On one occasion the veal was served in the form of a stew, while in the other case it was served roasted. Only one member

of the family knew the age of the veal. All appeared to enjoy the meat and made no remarks which would indicate that they were aware of its nature. No physiological disturbances were noted during these tests.

In general, it was noticed that when bob veal was cooked as a roast it presented a less appetizing appearance than did the more mature meats. This is due principally to the greater amount of water in the meat and to the less firm structure of the muscular tissue; consequently, when the meat is roasted or broiled—methods of cooking which cause the evaporation of considerable of the water—the meat shrinks away from the bones, producing an abnormal and undesirable appearance. However, if the veal is removed from the bone, it may be roasted, broiled, or used to make stews with very satisfactory results.

The younger veal was found to take the place quite satisfactorily of the common market veal. In practice, the shrinkage in cookery due to loss of water would mean the purchase of a larger quantity for the table if the same amount of meat is to be eaten. The deficiency in fat can be made up by adding fat in cookery. No study was made of the effects of handling upon market quality, or of the general question of legal regulation with respect to the marketing of young veal.

## DIGESTION EXPERIMENTS

### SELECTION AND COMPOSITION OF MATERIAL

The series of digestion experiments reported was made in this laboratory at the request of the Bureau of Animal Industry. The age of the calves used (in every case supplied by the Bureau of Animal Industry) was never more than five days, this age being arbitrarily selected in order to have as great a difference in maturity as possible between this type of veal and market veal. This was done so that any difference in the digestibility of the two types would be easier of detection should a difference exist. The calves used were procured without regard to breed or size and were healthy individuals passed by the Federal meat inspectors.

The calf was slaughtered the day preceding the cooking of the meat, and the carcass was stored in the meantime in a well-cooled refrigerator, no attempt being made, however, to study the keeping quality of the meat under ordinary trade and household conditions, a matter which apparently has not been studied. The cut most generally used in the digestion experiments was the leg, while the remainder was used for the dietary studies. This cut was chosen since it was easy to obtain the same cut of market veal for check experiments. The waste material (bone, tendon, etc.) in the legs of the very young veal was determined and found to be approximately 40 per cent. This amount of waste is much greater than that of mature veal, which is reported as 12 per cent (maximum, 25 per cent; 1, p. 31–32). Since muscular tissue is less developed in younger animals, it is logical to expect that there will be less tissue in proportion to bone.

In order to make a comparison of the percentages of the principal food constituents in the two types of uncooked meat, an analysis of the very young veal and market veal is given below. The percentages which are reported here are those obtained by averaging the results obtained from the analysis of a number of different samples. Very young veal: Water, 76.09 per cent; protein, 18.48 per cent; fat, 2.79 per cent; ash, 0.99 per cent. Market veal: Water, 71.97 per cent; protein, 20.07 per cent; fat, 7.43 per cent; ash, 1.28 per cent. A comparison of the two types of veal shows that very young veal contained more water than market veal and correspondingly less protein and fat. However, when the meat was cooked, the difference in the amount of protein and fat in the two types of veal was lessened, owing to the loss of more water from the very young veal than from the market veal. It was found that there was an average of 33.23 per cent of protein in the former and 34.41 per cent in the latter type of veal, although it is obvious that these figures represent merely the protein content of meat cooked by a single method. Obviously, if the meat were cooked in another way, for a longer or shorter period of time, using a different amount of fat, or employing more or less heat, the composition would vary quite materially. The chief difference in the composition of the meat from the very young and the older calves is in the percentage of water present; this decreases as the animal grows older, while at the same time the percentage of fat in the meat increases. Aside from this, the meat of the two ages shows very little difference.

## NATURE OF THE DIET

The very young veal was prepared by cutting in a meat cutter all the meat to be used for an experimental period. The meat was then thoroughly mixed to give a uniform product for eating and for analysis. After the meat had been prepared in this manner, it was cooked in the form of small cakes resembling Hamburg steak. A small amount of animal fat was used in cooking, but no attempt was made to increase materially the fat content of the meat cakes, and, roughly, the same amount was used for both bob veal and mature veal.

It has often been observed that the digestibility of a food is more satisfactorily determined if it be incorporated in a mixed diet than if eaten singly. Consequently, it was decided that the basal ration to be used in studying the digestibility of the meat in question should contain fruit, bread and butter, and tea or coffee with sugar, if desired. It can readily be seen that this diet contains all the essential constituents of a well-balanced ration, while at the same time the protein constituent of the diet is derived principally from the veal. It was impossible in these tests to prevent the subjects from knowing the nature of the diet. For instance, they all knew that they were having meat of some sort and that fat was used for the purpose of frying,

but it is hardly possible that they were definitely aware of the source of the meat. If this was the case, it is reasonable to believe that the appetite of the subjects and the digestibility of the food were not affected by any psychic factor.

The three-day or nine-meal experimental period, which is very often used in investigations of this kind, was again adjudged to be most satisfactory. In order that the subjects should experience no monotony while eating the ration, each test period of three days per week was followed by a rest period of four days; and, furthermore, the digestion experiments were conducted only on alternate weeks. During the intervening weeks tests were made of the digestibility of other food materials.

## SUBJECTS

Five subjects assisted in making this investigation. They were active young men of good physique and, as dental students, all were sufficiently interested in physiological questions to appreciate the importance of carrying out carefully such directions as were given them. They were urged to observe accuracy especially in the collection of feces, since in considering the digestibility of any food material it is more essential to know the amount of food retained and assimilated by the body than only the total amount of food consumed. To assist in identifying the feces of the test period, charcoal, which imparts a dark color to the feces, was given with the first meal of the test period and with the first meal following the period. The feces showing a dark color and all excreted until the dark color imparted by the charcoal was again noticed were retained for analytical purposes. The subjects were asked to bring notes describing their physical condition before, during, and after each test period. They all reported that with the exception of one or two colds they were in normal physical condition during the entire time that the investigation was in progress. Consequently, it has not been considered necessary to give in detail any of the individual reports which were received.

## EXPERIMENTS WITH VERY YOUNG VEAL

Inasmuch as lean meat like very young veal consists almost wholly of water and protein, these experiments are concerned only with the digestibility of protein. One method of determining the digestibility of a single food of a mixed diet is to determine by digestion experiments with the basal ration alone the amount of undigested residue occurring from the accessory foods, and for which a corresponding correction may be applied to the digestibility of the total ration. A second method consists in estimating the digestibility of the basal ration. Since the digestibility of the protein of wheat flour, fruit, and butter have been accurately determined by previous investigators, satisfactory factors are available

for estimating the digestibility of the protein furnished by these foods. Accordingly, in this investigation it has been assumed that the protein of bread is 93.8 per cent (8, p. 33), that of butter 97 per cent (2, p. 104), and that of fruit 85 per cent (2, p. 104) available. The following equations illustrate the method of applying the above factors:

[Weight of protein in bread, butter, fruit]×[Percentage of undigested protein in each]=[Undigested protein from basal ration].

[Total undigested protein]−[Undigested protein from basal ration]=[Undigested protein from meat alone].

[(Meat protein consumed)−(Undigested protein from meat)]÷[Meat protein consumed]=[Estimated percentage digestibility of meat protein].

The results which have been obtained in the tests of the digestibility of very young veal are given in Table I:

TABLE I.—*Data of digestion experiments with very young veal in a simple mixed diet*

| Item. | Weight. | Protein. | |
|---|---|---|---|
| | *Gm.* | *Gm.* | *Per cent.* |
| Experiment No. 5 (subject W. A. D.): | | | |
| Meat | 736.0 | 227.4 | 30.90 |
| Bread | 707.0 | 67.9 | 9.61 |
| Butter | 254.0 | 2.5 | 1.00 |
| Fruit | 1,467.0 | 4.3 | .29 |
| Total food consumed | 3,164.0 | 302.1 | ..... |
| Feces | 41.0 | 15.6 | 38.07 |
| Amount utilized | ..... | 286.5 | ..... |
| Digestibility of entire ration | ..... | ..... | 94.80 |
| Estimated digestibility of very young veal | ..... | ..... | 95.30 |
| Experiment No. 6 (subject E. D. J.): | | | |
| Meat | 704.0 | 217.5 | 30.90 |
| Bread | 942.0 | 90.7 | 9.63 |
| Butter | 207.0 | 2.1 | 1.00 |
| Fruit | 1,377.0 | 4.0 | .29 |
| Sugar | 202.0 | ..... | ..... |
| Total food consumed | 3,432.0 | 314.3 | ..... |
| Feces | 65.0 | 28.8 | 44.31 |
| Amount utilized | ..... | 285.5 | ..... |
| Digestibility of entire ration | ..... | ..... | 90.80 |
| Estimated digestibility of very young veal | ..... | ..... | 89.70 |
| Experiment No. 16 (subject J. H. K.): | | | |
| Meat | 703.0 | 287.6 | 40.91 |
| Bread | 745.0 | 66.5 | 8.93 |
| Butter | 154.0 | 1.5 | 1.00 |
| Fruit | 1,418.0 | 3.1 | .22 |
| Sugar | 310.0 | ..... | ..... |
| Total food consumed | 3,330.0 | 358.7 | ..... |

TABLE I.—*Data of digestion experiments with very young veal in a simple mixed diet*—Continued

| Item. | Weight. | Protein. | |
|---|---|---|---|
| Experiment No. 16—Continued. | *Gm.* | *Gm.* | *Per cent.* |
| Feces | 45. 0 | 21. 6 | 48. 50 |
| Amount utilized | ..... | 337. 1 | ..... |
| Digestibility of entire ration | ..... | ..... | 94. 00 |
| Estimated digestibility of very young veal | ..... | ..... | 94. 10 |
| Experiment No. 17 (subject W. E. L.): | | | |
| Meat | 706. 0 | 289. 1 | 40. 95 |
| Bread | 816. 0 | 72. 8 | 8. 92 |
| Butter | 129. 0 | 1. 3 | 1. 00 |
| Fruit | 1, 557. 0 | 3. 5 | . 22 |
| Sugar | 188. 0 | ..... | ..... |
| Total food consumed | 3, 396. 0 | 366. 7 | ..... |
| Feces | 44. 0 | 18. 9 | 42. 94 |
| Amount utilized | ..... | 347. 8 | ..... |
| Digestibility of entire ration | ..... | ..... | 94. 80 |
| Estimated digestibility of very young veal | ..... | ..... | 95. 40 |
| Experiment No. 27 (subject W. A. D.): | | | |
| Meat | 699. 0 | 202. 3 | 28. 94 |
| Bread | 859. 0 | 76. 8 | 8. 94 |
| Butter | 133. 0 | 1. 3 | 1. 00 |
| Fruit | 1, 218. 0 | 3. 0 | . 25 |
| Total food consumed | 1, 909. 0 | 283. 4 | ..... |
| Feces | 56. 0 | 11. 4 | 20. 37 |
| Amount utilized | ..... | 272. 0 | ..... |
| Digestibility of entire ration | ..... | ..... | 96. 00 |
| Estimated digestibility of very young veal | ..... | ..... | 97. 30 |
| Experiment No. 28 (subject J. R. F.): | | | |
| Meat | 752. 0 | 217. 6 | 28. 94 |
| Bread | 717. 0 | 64. 1 | 8. 94 |
| Butter | 206. 0 | 2. 1 | 1. 00 |
| Fruit | 1, 209. 0 | 3. 0 | . 25 |
| Sugar | 68. 0 | ..... | ..... |
| Total food consumed | 2, 952. 0 | 286. 8 | ..... |
| Feces | 58. 0 | 23. 3 | 40. 22 |
| Amount utilized | ..... | 263. 5 | ..... |
| Digestibility of entire ration | ..... | ..... | 91. 90 |
| Estimated digestibility of very young veal | ..... | ..... | 91. 40 |
| Experiment No. 29 (subject W. E. L.): | | | |
| Meat | 684. 0 | 197. 9 | 28. 94 |
| Bread | 683. 0 | 61. 1 | 8. 94 |
| Butter | 127. 0 | 1. 3 | 1. 00 |
| Fruit | 1, 280. 0 | 3. 2 | . 25 |
| Sugar | 115. 0 | ..... | ..... |
| Total food consumed | 2, 066. 0 | 263. 5 | ..... |

TABLE I.—*Data of digestion experiments with very young veal in a simple mixed diet*—Continued

| Item. | Weight. | Protein. | |
|---|---|---|---|
| Experiment No. 29—Continued. | *Gm.* | *Gm.* | *Per cent.* |
| Feces | 68. 0 | 32. 5 | 47. 82 |
| Amount utilized | ..... | 231. 0 | ..... |
| Digestibility of entire ration | ..... | ..... | 87. 70 |
| Estimated digestibility of very young veal | ..... | ..... | 85. 80 |
| Average food consumed per subject per day | 964. 2 | 103. 6 | ..... |

SUMMARY OF EXPERIMENTS WITH VERY YOUNG VEAL

| Experiment No. | Subject. | Digestibility of protein. | |
|---|---|---|---|
| | | Total diet. | Veal alone. |
| | | *Per cent.* | *Per cent.* |
| 5 | W. A. D. | 94. 8 | 95. 3 |
| 6 | E. D. J. | 90. 8 | 89. 7 |
| 16 | J. H. K. | 94. 0 | 94. 1 |
| 17 | W. E. L. | 94. 8 | 95. 4 |
| 27 | W. A. D. | 96. 0 | 97. 3 |
| 28 | J. R. F. | 91. 9 | 91. 4 |
| 29 | W. E. L. | 87. 7 | 85. 8 |
| | Average | 92. 9 | 92. 7 |

From the data recorded in these tables it may be calculated that an average of 237 gm. of meat, furnishing 78 gm. of protein, or 75 per cent of the total protein of the diet, was eaten daily. The five subjects completed the experiments in good condition and without having experienced any physiological disturbances. The average values of seven experiments for the digestibility of total protein and that of meat protein alone are practically identical, being for the former 92.9 per cent and for the latter 92.7 per cent. The values estimated for the digestibility of bob-veal protein in the different experiments are not consistently higher or lower than the determined values for the protein of the total diet. This irregularity is very likely due to the variation in the amounts of protein obtained from the different sources.

## CHECK EXPERIMENTS

Tests of the digestibility of market veal, using the same basal ration and with the same subjects and method of cooking as for bob veal, were made in order to compare the digestibility of very young and market veal under identical conditions. For this purpose legs of veal from animals at least 4 weeks old were purchased in the open market. Although it was realized that market veal contained a larger percentage

of fat than bob veal, only the superficial fat was removed before cooking, no attempt being made to secure meat cakes from market veal of the same composition as those made of bob veal. The fat contained in the market veal comprised the minor portion of the total fat content of the diet, and, moreover, for the sake of comparison with bob veal, it was necessary to know only the digestibility of protein. The digestibility of the protein alone, therefore, has been studied in these experiments. This is reported for the entire ration and has been estimated for the protein of meat alone in the manner previously described.

The results of three experiments with three subjects are given in Table II.

TABLE II.—*Data of digestion experiments with market veal in a simple mixed diet*

| Item. | Weight. | Protein. | |
|---|---|---|---|
| | *Gm.* | *Gm.* | *Per cent.* |
| Experiment No. 18 (subject J. H. K.): | | | |
| Meat | 629.0 | 219.0 | 34.81 |
| Bread | 569.0 | 53.0 | 9.31 |
| Butter | 178.0 | 1.8 | 1.00 |
| Fruit | 1,568.0 | 3.0 | 0.19 |
| Total food consumed | 2,944.0 | 276.8 | ..... |
| Feces | 38.0 | 17.1 | 45.00 |
| Amount utilized | ..... | 259.7 | ..... |
| Digestibility of entire ration | ..... | ..... | 93.80 |
| Estimated digestibility of market veal | ..... | ..... | 93.90 |
| Experiment No. 19 (subject W. E. L.): | | | |
| Meat | 689.0 | 239.8 | 34.81 |
| Bread | 725.0 | 67.5 | 9.31 |
| Butter | 158.0 | 1.6 | 1.00 |
| Fruit | 1,536.0 | 2.9 | 0.19 |
| Sugar | 156.0 | ..... | ..... |
| Total food consumed | 3,264.0 | 311.8 | ..... |
| Feces | 57.0 | 24.8 | 43.5 |
| Amount utilized | ..... | 287.0 | ..... |
| Digestibility of entire ration | ..... | ..... | 92.00 |
| Estimated digestibility of market veal | ..... | ..... | 91.60 |
| Experiment No. 22 (subject W. A. D.): | | | |
| Meat | 653.0 | 277.9 | 42.56 |
| Bread | 801.0 | 59.6 | 7.44 |
| Butter | 92.0 | 0.9 | 1.00 |
| Fruit | 1,702.0 | 4.3 | 0.25 |
| Total food consumed | 3,248.0 | 342.7 | ..... |
| Feces | 62.0 | 24.1 | 38.94 |
| Amount utilized | ..... | 318.6 | ..... |
| Digestibility of entire ration | ..... | ..... | 93.00 |
| Estimated digestibility of market veal | ..... | ..... | 92.90 |
| Average food consumed per subject per day | 1,050.7 | 103.4 | ..... |

SUMMARY OF EXPERIMENTS WITH MARKET VEAL

| Experiment No. | Subject. | Digestibility of protein. | |
|---|---|---|---|
| | | Total diet. | Market veal alone. |
| | | *Per cent.* | *Per cent.* |
| 18 | J. H. K. | 93.8 | 93.9 |
| 19 | W. E. L. | 92.0 | 91.6 |
| 22 | W. A. D. | 93.0 | 92.9 |
| | Average | 92.9 | 92.8 |

The digestibility of the protein of the total diet was determined to be 92.9 per cent, while it was estimated that the protein of market veal alone was 92.8 per cent available, values somewhat lower than those found by Grindley (7) for roast veal and for meat in general. The amounts of food eaten in these experiments and those with bob veal were approximately the same and furnished the same average amount of total protein (103 gm. per day), indicating that both rations were eaten with equal relish.

## CONCLUSIONS

As determined by the experiments herein reported, the digestibility of the protein of bob veal is the same as that found for market veal—namely, 93 per cent, in round numbers. The subjects of both dietary and digestion experiments, so far as could be learned, experienced no physiological disturbances during the experimental period or afterwards. The tests showed that such veal can be prepared for the table in palatable ways and that so far as could be judged it was not unwholesome when eaten in quantity. In the digestion experiments the average weight of protein supplied by the meat exceeded that generally furnished by the meat portion of the ordinary diet, indicating that very young or bob veal was not distasteful. The experiments here reported also indicate that the general opinion that young veal is a common cause of digestive disturbance or fails to digest as thoroughly as similar foods is not justified.

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# INFLUENCE OF CALCIUM AND MAGNESIUM COMPOUNDS ON PLANT GROWTH[1]

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## INTRODUCTION

Some investigators seem to question the advisibility of using magnesium-bearing minerals in agricultural practices, since they deem magnesium detrimental to optimum plant growth. Magnesium in some forms is detrimental to plant growth. However, the natural carbonates, such as limestones and dolomites, are not detrimental but in reality beneficial to plant growth when applied in amounts sufficient to neutralize soil acidity. Plants were found to grow and mature normally in pure dolomite and limestone.

In scientific circles considerable attention has been paid to the theory that calcium and magnesium must occur in a definite ratio for the optimum production of crops. Loew claims to have proposed this theory in 1892 (15)[3], and much work has been conducted along this line, especially during the last decade. From the data presented in the following pages it will be seen that the ratio, within wide limits, had no effects.

The presence of sufficient quantities of calcium and magnesium in all soils is essential for the profitable production of crops. Various forms and quantities of these two elements may largely control the yields and composition of the harvests.

It is a well-known fact that plants will tolerate larger amounts of an essential element than they require. The quantity of calcium and magnesium taken up by plants is dependent upon the amount available and upon the kind of plants. The silicates of calcium and magnesium are relatively insoluble, while the chlorids are very soluble. Dolomite is denser and less soluble than limestone but more soluble than magnesite. Synthetic compounds of magnesium are more soluble, however, than similar compounds of calcium.

Alfalfa, when grown in sand and soil cultures with varying amounts of calcium and magnesium minerals, such as dolomite and magnesite, also with prepared compounds of these two elements, such as the chlorids, sulphates, and carbonates, was found to contain varying amounts of

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[3] Bibliographic citations in parentheses refer to "Literature cited," p. 616–619.

calcium and magnesium. Some treatments showed as much as 52.5 pounds of calcium and 12.98 pounds of magnesium per ton of dry alfalfa. However, the above amounts were in excess of the absolute requirements, as smaller applications gave as large yields and the alfalfa contained only 28 pounds of calcium and 8 pounds of magnesium per ton of dry matter. On this basis 6 tons of alfalfa with a high-calcium content would contain 315 pounds of calcium and 77.88 pounds of magnesium, or the equivalent of 787.5 pounds of calcium carbonate and 272.5 pounds of magnesium carbonate. Wheat straw, when grown in pure dolomite, contained 14.48 pounds of calcium and 14.6 pounds of magnesium per ton, whereas when grown in the absence of excessive amounts of these two elements the straw contained only 5.96 pounds of calcium and 5.43 pounds of magnesium per ton.

## REVIEW OF THE LITERATURE

Solution cultures and pot cultures have contributed largely to our present knowledge of plant nutrition. Woodward (42) found that the solid particles of the soil furnished nourishment to the growing plants and that water acted only as a carrier.

Wolf (4) found by using beans and maize in controlled solutions, that the concentration as well as the kind of salts in the solution effected plant growth. His results show that when the concentration of the external solution was more than 0.25 per cent it became the controlling factor; whereas if less than 0.25 per cent absorption was controlled by the solution within the roots.

Dassonville (1) found that cutinization and lignification of the epidermis of leaves occurred much more rapidly in distilled water than in nutrient solutions; also that the growth of hemp and buckwheat was not influenced by the presence or absence or calcium and magnesium.

The crop is the measure of the resultant of all factors. In accordance with the present knowledge any one or many of the factors can be controlled. Likewise, the total amounts of the elements essential for crop production can be quantitatively determined.

Magnesium is essential for the growth of any living cell. Calcium is likewise essential except for the lower fungi and lower algæ, which alone are able to exist without it. Loew (16, p. 44) shows that neutral oxalates are not poisonous to the lower fungi. He attributes the deleterious effects in higher plants to the change in the structure of the calcium-protein compounds, due to the formation of calcium oxalate, while the disturbance is brought about by the change in imbibition caused by the formation of potassium-protein compounds, and that magnesium may bring about this change provided there is a deficiency in calcium.

Reed (29) found calcium to be necessary to the activity and growth of chlorophyll-containing organs. Willstätter (40) has pursued in detail

the study of chlorophyll and finds it to be a magnesium compound with generally three times as much green pigment as yellow pigment. He found that the magnesium content of chlorophyll was constant in both land and sea plants; therefore, it must function other than as a catalyzer. Pfeffer (27, p. 425), Macdougall (17, p. 219), Peirce (26, p. 100) and others believe magnesium and calcium play an important and necessary function in plant synthesis and cell formation, but are unable to assign any specific rôle to either of these elements.

There has been considerable contention as to whether calcium could be replaced by other members of the group. Haselhoff (7) grew beans and maize in solutions containing varying quantities of calcium and strontium and concluded that strontium seemed to take the place of calcium, replacing it only when the supply of calcium was inadequate. But it must be remembered that he first used calcium and strontium together in the solution and later reduced the calcium. However, Loew (16, p. 48) was unable to substantiate these results when he used species of Tradescantia.

Loew explains the toxicity between calcium and magnesium as being due to the formation of an insoluble condition of the phosphoric acid being fixed by the calcium, and that the framework (15) of the nucleus and plastids is a double organic salt of calcium and magnesium. However, Meurer (19) and Nathansohn (23) offer another explanation: Cells being selective in their absorption of ions can check osmosis before a balance is reached between the solution within and without the cell, and the absorption of salts does not increase proportionally with the increase of concentration of the outside solution. Osterhout (24) using calcium nitrate and magnesium nitrate was unable to substantiate Loew's assertion.

Considerable work has been done upon the antagonism of respective salts for each other in solution. Kearney (11, p. 20) shows that calcium salts are most beneficial in reducing toxicity. Lipman (14) reports toxicity between magnesium and sodium but not between magnesium and calcium.

Numerous investigators have sought answers to the proposed theory of a lime-magnesia ratio with just as numerous and conflicting results. Solutions, pot cultures of soil and sand, and field soils have all been employed in attempts to settle the controversy. Ulbricht (34) showed that yellow lupines, barley, and vetch were injured by applications of lime, especially when it contained high percentages of magnesia. Magnesia apparently increased the proportional yield of grain in the case of barley and lupines. Dojarenko (2), however, concluded that the theory of a definite calcium-magnesium ratio was not tenable, as many Russian soils containing great excesses of calcium over magnesium were benefited by liming.

The results of water and soil cultures by Gössel (6) failed to substantiate the theory of a definite ratio of calcium to magnesium. He obtained the highest yields for beans and barley with water cultures when the ratio of lime to magnesia was 0.04 to 1, and concluded that the effect of liming is dependent upon the character of the soil and not upon a definite ratio of lime to magnesia. About this same time the Japanese investigators (22) were actively engaged with the problem. However, their results all seem to bear out the theory of a definite ratio.

Konovalov (12), a Russian investigator, reports studies with barley, millet, oats, and maize, varying the ratio of calcium oxid to magnesium oxid, as follows: 13.4 to 1, 6.7 to 1, 3.3 to 1, 0.8 to 1, and 0.4 to 1. He found that the yields tended to increase with the increase of lime application, provided the magnesia content remained constant. Notwithstanding these results, Voelcker (35, 36, 37) states that the ratio is best at 1 to 1.

Meyer (20, 21) found that with buckwheat and oats the dependence of maximum yields on a definite ratio of calcium to magnesium could not be proved even in the case of soils containing more calcium than magnesium or vice versa. Undoubtedly the most extensive investigations regarding a definite calcium-magnesium ratio have been conducted by Lemmerman (13) et al. They used six different soils and grew vetch, oats, barley, rye, wheat, clover, mustard, and buckwheat, with the investigations extending over three years, 1907 to 1909, inclusive. From the standpoint of yields the ratio had no effects within wide limits. Stewart (33) reports soils having 16.88 per cent of calcium oxid and 6.1 per cent of magnesium oxid which were cropped for 40 years without the addition of fertilizers, except in the case of sugar beets, which received manure. The 8-year average yields are 80 bushels for oats, 50.4 bushels for wheat, 262.3 bushels for potatoes, and 21.8 tons for sugar beets.

Wartiadi (38) used sand and water cultures with wheat and barley and found that calcium and magnesium were beneficial or detrimental in proportion to their relative amounts in the culture solution. Russell (30, p. 144) finds no connection between the lime-magnesia ratio and the productivity of the soil. Haselhoff (8) also failed to substantiate Loew's theory, while Hopkins (9, pp. 170–171) found magnesium carbonate beneficial up to 0.8 per cent when added alone and in connection with calcium sulphate in such amounts as to maintain a ratio of 4 to 7, respectively, of magnesium oxid and calcium oxid.

Gile (3) reports that with the chlorids of calcium and magnesium at low concentrations the ratio exerted no influence, while at high concentrations it was effective. Good yields of pineapples (4) were produced from soils in Porto Rico when the ratio varied between 1 to 13 and 73 to 1; and in one field where the ratio of calcium oxid to magnesium oxid was 1,461 to 1 a yield of 60 tons of sugar cane was realized.

Pisciotta (28), an Italian investigator, reports the analysis of 60 soils which show a wide variation in the lime-magnesia ratio, due to the variation in the lime content. Patterson (25) found that magnesian lime, which is claimed to be poisonous, gave the highest yields.

In summing up the literature studies previously mentioned, it will be seen that Loew and his associates and Japanese students maintain the theory of a definite lime-magnesia ratio, as do Ulbricht and Wartiadi, whereas Dojarenko, Gössel, Konovalov, Meyer, Lemmerman, Haselhoff, Gile, and Patterson claim that a definite ration of lime to magnesia is not tenable and, furthermore, lacks substantiation.

Lemmerman et al. have undoubtedly conducted the most extensive investigations upon this subject and conclude that there is no correlation between maximum crop productions and the ratio of lime to magnesia. Soils reported by Russell and by Gile show wide variations in the lime-magnesia ratio, also in the percentages of these two elements, and that there fails to be any correlation between the productivity of a soil and its ratio of lime to magnesia.

Solution cultures show that a specific ratio of lime to magnesia is not equally effective in dilute solutions and in concentrated solutions. This indicates that the effectiveness is dependent upon the total balance of all the salts in solution instead of merely the ratio of calcium to magnesium.

The preponderance of evidence appears to be against a definite ratio of lime to magnesia, especially with respect to soil cultures in pots and under field conditions. What really seems of first magnitude is the resultant of all factors—that is, the climate, the plant, and structure, reaction, micro-organic activity, and composition of the soil.

## EXPERIMENTAL WORK

These experiments were planned with the idea of studying the effects of calcium and magnesium upon plant growth when applied in different natural and in artificially prepared forms. Studies were made to determine the amount of calcium and magnesium which the plants could tolerate. The relation between the ratios of these two elements in the plants, in the soils, and in the materials applied was also studied.

Dolomite, limestone, magnesite, calcareous soils, and brown silt loam were used as sources of the natural forms, while prepared materials, such as the carbonates, chlorids, and sulphates, served as sources of the artificial forms. Increasing amounts of the various forms were used, also a variance in the ratio of calcium to magnesium was employed. The earlier applications varied from 0.1 to 0.6 per cent of magnesium added in magnesium carbonate and in magnesite. Later the following amounts were employed: 2, 6, and 10 per cent of magnesium in magnesite; 10 and 12.7 per cent of magnesium in dolomite; 0.1, 0.01, and 0.001 per cent of magnesium in the carbonates, chlorids, and sulphates. In each series sand or soil was used as a control.

## DESCRIPTION OF PROCEDURE AND METHODS

Earthen pots 6.5 inches in diameter by 7.5 inches in depth were used. Each pot contained 13.2 pounds of sand, while in the soil series each contained 8.8 pounds of brown silt loam. Sand and soil were used as mediums of control, and to these two materials were added the various forms and amounts of calcium and magnesium.

Various methods were pursued in extracting the sand. At first dilute hydrochloric acid (HCl) was kept in contact with the sand for 48 hours, but this failed to remove all the calcium and magnesium. Later the sand was extracted with stronger acid (1,350 c. c. of concentrated hydrochloric acid plus 1,000 c. c. of distilled water) for periods of from 9 to 14 days. Sand was also digested on a steam bath for 4 days with this same strength acid. None of the above processes were able to remove all the calcium and magnesium from the sand, as will be seen from the analysis reported.

At intervals varying from 10 days to 2 weeks plant food was added from the following solutions:

(1) Potassium sulphate, 50 gm. to 2½ liters of water; (2) ammonium nitrate, 80 gm. to 2½ liters of water; (3) disodium phosphate, 26.1 gm. to 2½ liters of water; (4) ferric chlorid, 0.4 gm. to 1 liter of water.

The calcium and magnesium were applied in forms previously mentioned. The moisture content of the sand was at first 12 per cent, but it was later raised to 14 per cent, while for the brown silt loam it was 24 per cent. Every 10 days the pots were brought to standard weight by adding distilled water.

All crops were grown in the agronomy greenhouses at the University of Illinois. The principal crops used in these studies were wheat (*Triticum* spp.), alfalfa (*Medicago sativa*), soybeans (*Soja max*), and cowpeas (*Vigna sinensis*). Oats (*Avena sativa*), clover (*Trifolium pratense*), timothy (*Phleum pratense*), and sweet clover (*Melilotus alba*) were also used to test the effect of artificial carbonates upon germination.

In the wheat and soybean series, 10 seeds per pot were planted and 7 plants permitted to grow, while for alfalfa 15 plants were permitted to grow whenever possible.

In making determinations for calcium and magnesium, the soils were first fused with sodium peroxid and from this point the usual method was employed. The calcium oxalate was dissolved in dilute sulphuric acid ($H_2SO_4$) and the calcium calculated from the amount of *N/10* potassium permanganate required to oxidize the oxalic acid thus formed. The magnesium was precipitated as magnesium-ammonium phosphate and burned to the pyrophosphate. In analyzing the plants 2 gm. of finely ground material were ashed, taken up in hydrochloric acid, and the calcium and magnesium determined as above stated. Acid extractions of the dolomites and limestones proved as good as fusions.

TABLE I.—*Composition of materials supplying calcium and magnesium to the soil*

| Material. | Calcium carbonate. | Magnesium carbonate. | Calcium. | Magnesium. | Molecular ratio of calcium to magnesium. |
|---|---|---|---|---|---|
| | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | |
| Dolomite C1 | 43.65 | 35.26 | ..... | ..... | 5:4.8 |
| Dolomite C3 | 51.18 | 44.9 | ..... | ..... | 5:5.2 |
| Magnesite C4 | ..... | 98.37 | ..... | ..... | ..... |
| Limestone [1] | 84.2 | 5.85 | ..... | ..... | ..... |
| Brown silt loam | ..... | ..... | 0.305 | 0.35 | 5:9.6 |
| Calcareous soil | ..... | ..... | 5.58 | 2.64 | 5:3.8 |
| Sand extracted with dilute hydrochloric acid for 48 hours | ..... | ..... | .0142 | .016 | ..... |
| Sand extracted with concentrated hydrochloric acid for four days on a steam bath | ..... | ..... | .0128 | .0089 | ..... |
| Sand not extracted | ..... | ..... | .017 | .0148 | ..... |

[1] From Columbia, Ill.

The brown silt loam used in series C and D was taken from the surface of sod land and had only the coarser roots removed. The calcareous soil was taken from a layer varying from 8 to 12 inches in thickness and 72 inches below the surface in the forestry near plot 719 of the north farm of the university. The coarser pebbles were removed before using. The analysis in Table I refers to the portion used in growing crops; the coarser pebbles show a higher content of calcium and magnesium, or 13.34 per cent of calcium and 5.97 per cent of magnesium.

The 6,000 gm. of sand, after being extracted with hydrochloric acid, contained for each pot from 768 to 852 mgm. of calcium and from 540 to 960 mgm. of magnesium. From the following tables it will be seen that the plants had the power to obtain considerable quantities of the apparently insoluble calcium and magnesium silicate, as they obtained quantities in excess of the amount added in the seeds, although they show lower contents than plants grown in an excess of these materials. The chemically pure magnesium carbonate gave an immediate alkaline reaction to phenolphthalein upon the addition of distilled water. Dolomites C1 and C3 likewise showed an alkaline reaction to this indicator, but only after having been in distilled water over night. Magnesite after standing in distilled water from 8 to 12 hours was alkaline to phenolphthalein, as was also the calcareous soil.

In experimenting with artificially prepared magnesium carbonate, a great deal of care was taken to obtain the alkali-free substance to begin with. This can be prepared by a precipitation from solution with ammonium carbonate. Magnesium carbonate in the presence of water has a great tendency to hydrolyze, which may at least partially explain its poisonous effect.

EFFECT OF MAGNESIUM AND CALCIUM IN PREPARED CARBONATES AND IN DOLOMITE UPON WHEAT AND ALFALFA GROWN IN SAND (SERIES A AND B)

The sand used in series A and B was extracted as previously described, washed free from acid, and the application made on the moisture-free basis. Pots 1 and 2 of series A and pots 21 and 22 of series B received no calcium or magnesium. Pots 3 and 4 of series A and pots 23 and 24 of series B received 2 per cent of dolomite C1 or 0.2 per cent of the element magnesium. Pots 9 to 20, inclusive, of series A and pots 29 to 40, inclusive, of series B received magnesium and calcium in prepared carbonate in amounts varying from 0.1 per cent to 0.6 per cent of magnesium. The magnesium carbonate was alkaline and of the following formula: $Mg(OH)_2.4MgCO_3$.

Table II contains the yields for the above treatments. When added to sand, the lowest applications of magnesium in the prepared carbonate very materially retarded germination and inhibited growth; whereas amounts of 0.6 per cent of magnesium in dolomite caused no injury and even benefited growth.

The ratio of calcium to magnesium throughout this and all succeeding tables is reported as molecular calcium to molecular magnesium, with calcium always expressed as 5. The yields and percentages in all the tables are reported on the water-free basis. The wheat, series A, was harvested 83 days after being planted, while the alfalfa, series B, was harvested 84 days after being planted.

TABLE II.—*Yields of wheat and alfalfa (in grams per pot on a water-free basis) in sand—series A and B*

| Treatment. | Molecular ratio of calcium to magnesium. | Wheat, series A. | | | Alfalfa, series B. | | |
|---|---|---|---|---|---|---|---|
| | | Pot No. | Tops. | Roots. | Pot No. | Tops. | Roots. |
| None | ..... | 1 | 20.9 | 22.2 | 21 | 5.16 | 6.42 |
| None | ..... | 2 | 18.5 | 23.2 | 22 | 3.12 | 2.58 |
| Percentage of magnesium in dolomite C1: | | | | | | | |
| 0.2 | 5:4.8 | 3 | 7.07 | 5.4 | 23 | 6.24 | 8.73 |
| .2 | 5:4.8 | 4 | 23.0 | 18.8 | 24 | 7.84 | 12.47 |
| .4 | 5:4.8 | 5 | 23.0 | 15.0 | 25 | 5.97 | 9.0 |
| .4 | 5:4.8 | 6 | 17.8 | 10.0 | 26 | 5.61 | 8.02 |
| .6 | 5:4.8 | 7 | 20.7 | 12.9 | 27 | 7.75 | 10.34 |
| .6 | 5:4.8 | 8 | 10.8 | 6.3 | 28 | 7.21 | 9.0 |

The pots receiving magnesium and calcium in the prepared carbonates in a ratio of calcium to magnesium, as 5 to 4, inhibited germination and permitted no growth.

Table III shows the analysis of the plants reported in Table II. The results here reported are the average of four determinations from duplicate pots.

From Table III it can be seen that the alfalfa is a heavier feeder on calcium and magnesium than is wheat, and that the percentages and the total amounts removed by the plants tend to increase with the increase in application, except where the calcium and magnesium are applied in the artificially prepared carbonates, in which case the lowest application is sufficient to inhibit growth and retard germination.

Throughout all the series the general tendency was for the calcium and magnesium content of the plants to increase with the increase in application. Wheeler (39) found that when magnesium was applied in the form of the sulphate the crop showed the ratio of magnesium oxid to calcium oxid to be as 1 to 1.13, but when magnesium was not present in the fertilizer the ratio of magnesium oxid to calcium oxid was 1 to 2.7.

TABLE III.—*Analyses of wheat and alfalfa—series A and B*

| Pot No. | Substance added. | | Composition of plants. | | | | |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium. | Calcium. | | Magnesium. | | Molecular ratio of calcium to magnesium. |
| | *Per cent.* | *Per cent.* | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| **WHEAT STRAW** | | | | | | | |
| 1 and 2 [a] | 0.0142 | 0.0165 | 37.0 | 0.187 | 21.4 | 0.108 | 5:5.1 |
| 3 | .333 | .2 | 68.5 | .298 | 62.6 | .271 | 5:7.6 |
| 7 and 8 | .999 | .6 | 60.1 | .386 | 66.6 | .421 | 5:9.1 |
| **WHEAT ROOTS** | | | | | | | |
| 1 and 2 | .0142 | .0165 | 43.4 | .187 | 24.8 | .09 | 5:4.1 |
| 3 | .333 | .2 | 85.3 | .452 | 55.3 | .292 | 5:5.4 |
| 7 and 8 | .999 | .6 | 91.8 | .96 | 47.9 | .50 | 5:4.35 |
| **ALFALFA HAY** | | | | | | | |
| 21 and 22 [a] | .0142 | .0165 | 14.4 | .347 | 6.84 | .164 | 5:3.9 |
| 23 and 24 | .333 | .2 | 181.0 | 2.565 | 30.6 | .431 | 5:1.4 |
| 27 and 28 | .999 | .6 | 196.5 | 2.622 | 48.6 | .649 | 5:2 |
| **ALFALFA ROOTS** | | | | | | | |
| 21 and 22 | .0142 | .0165 | 8.52 | .19 | 6.26 | .14 | 5:6.1 |
| 23 and 24 | .333 | .2 | 48.8 | .46 | 50.0 | .472 | 5:8.5 |
| 27 and 28 | .999 | .6 | 54.4 | .561 | 78.9 | .815 | 5:12 |

[a] Pots 1, 2, 21, and 22 were extracted sand and received no calcium and magnesium.

EFFECTS OF CALCIUM AND MAGNESIUM IN PREPARED CARBONATES AND IN DOLOMITE UPON WHEAT AND ALFALFA IN BROWN SILT LOAM (SERIES C AND D)

Magnesium and calcium in prepared carbonates were less harmful in brown silt loam than in sand (series C and D). In fact, applications of 0.1 per cent of magnesium or 0.35 per cent of magnesium carbonate gave an increase over the check, and 0.7 per cent of the carbonate was practically as good. It must be remembered that the soil before treatment contained 0.305 per cent of calcium and 0.352 per cent of magnesium. The calcium and magnesium were added in the relation of 5 to 4, but the amounts in the soil changed this ratio to 5 to 7.1. Applications of 3.5 tons of prepared magnesium carbonate per acre were beneficial, 7 tons were about equal to the check, while upward of 10 tons caused practically no growth of the plants.

TABLE IV.—*Yields of wheat and alfalfa (in grams per pot on a water-free basis) in brown silt loam—series C and D*

| Treatment. | Molecular ratio of calcium to magnesium. | Wheat, series C. | | | Alfalfa, series D. | | |
|---|---|---|---|---|---|---|---|
| | | Pot No. | Tops. | Roots. | Pot No. | Tops. | Roots. |
| Brown silt loam only | 5:9.6 | 41 | 8.9 | 6.8 | 65 | 4.64 | 6.86 |
| Do | 5:9.6 | 42 | 9.3 | 7.9 | 66 | 4.28 | 6.53 |
| Percentage of magnesium in magnesium carbonate plus calcium carbonate: | | | | | | | |
| 0.1 | 5:7.1 | 43 | 11.6 | 7.16 | 67 | 5.96 | 6.95 |
| | 5:7.1 | 44 | 11.0 | 7.0 | 68 | 5.35 | 6.5 |
| .2 | 5:7.1 | 45 | 8.1 | 5.55 | 69 | 2.58 | 2.49 |
| | 5:7.1 | 46 | 8.1 | 6.07 | 70 | 3.03 | 2.85 |
| .3 | 5:7.1 | 47 | 1.45 | 1.81 | 71 | .09 | ..... |
| | 5:7.1 | 48 | 4.08 | 3.17 | 72 | 2.58 | 1.69 |
| .4 | 5:7.1 | 49 | .9 | .72 | 73 | ..... | ..... |
| | 5:7.1 | 50 | .18 | .18 | 74 | ..... | ..... |
| .5 | 5:7.1 | 51 | 1.85 | 2.35 | 75 | ..... | ..... |
| | 5:7.1 | 52 | .09 | .09 | 76 | ..... | ..... |
| .6 | 5:7.1 | 53 | .09 | .09 | 77 | ..... | ..... |
| | 5:7.1 | 54 | .09 | ..... | 78 | ..... | ..... |
| Percentage of magnesium in dolomite C1: | | | | | | | |
| 0.2 | 5:4.8 | 55 | 9.52 | 5.45 | 79 | 5.17 | 6.05 |
| | 5:4.8 | 56 | 10.5 | 6.07 | 80 | 5.0 | 5.08 |
| .4 | 5:4.8 | 57 | 9.61 | 8.15 | 81 | 5.35 | 6.85 |
| | 5:4.8 | 58 | 9.07 | 7.25 | 82 | 5.08 | 5.88 |
| .6 | 5:4.8 | 59 | 10.0 | 8.8 | 83 | 4.37 | 3.74 |
| | 5:4.8 | 60 | 9.52 | 8.15 | 84 | 5.43 | 6.15 |
| 2 | 5:4.8 | 61 | 8.9 | 4.53 | 85 | 5.43 | 4.72 |
| | 5:4.8 | 62 | 5.71 | 4.62 | 86 | 5.60 | 4.64 |
| 4 | 5:4.8 | 63 | 6.07 | 4.53 | 87 | 5.17 | 5.08 |
| | 5:4.8 | 64 | 7.52 | 4.53 | 88 | 5.52 | 3.65 |

MacIntire (18), while working with three different kinds of soils, found that 8 tons per acre of precipitated magnesium carbonate were decidedly toxic to wheat. He also found that both the oxids and the carbonates of precipitated magnesium were many times more soluble than the corresponding forms of calcium, while in the case of the native mineral

carbonates limestone was 1.62 times as soluble as dolomite and more than 3 times as soluble as magnesite.

Applications of dolomite C1 up to 40 per cent caused no injury to either wheat or alfalfa.

Table IV shows the treatments and yields of series C and D. Analyses of these plants are reported in Table V. Series C (wheat) was harvested 83 days after planting and series D (alfalfa) was harvested 84 days after planting.

From Table V it can be seen that wheat grown in soil (pots 41 and 42) shows 0.279 per cent of calcium and 0.256 per cent of magnesium, but when grown in extracted sand (Table III, pots 1 and 2) it had only 0.187 per cent of calcium and 0.108 per cent of magnesium, showing that there is a decided tolerance for these two elements. By comparing the wheat with the alfalfa it can be seen that, while alfalfa is a heavier feeder than wheat on calcium and magnesium, the proportional amounts of calcium are greater in alfalfa than in wheat.

TABLE V.—*Analysis of wheat and alfalfa grown in brown silt loam—series C and D*

| Pot No. | Substance added. | | Composition of plants. | | | | |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium. | Calcium. | | Magnesium. | | Molecular ratio of calcium to magnesium. |
| | *Per cent.* | *Per cent.* | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| **WHEAT STRAW** | | | | | | | |
| 41 and 42 | 0.305 | 0.352 | 27.1 | 0.297 | 23.31 | 0.256 | 5: 7.2 |
| 43 and 44 | .527 | .458 | 33.6 | .297 | 52.7 | .467 | 5:13.1 |
| 47 and 48 | .971 | .671 | 9.76 | .352 | 32.8 | 1.19 | 5:28.1 |
| 55 and 56 | .315 | .2 | 35.4 | .353 | 33.8 | .337 | 5: 7.9 |
| 63 and 64 | 6.30 | 4.0 | 29.2 | .43 | 31.0 | .456 | 5 8.8 |
| **WHEAT ROOTS** | | | | | | | |
| 41 and 42 | .305 | .352 | 21.9 | .298 | 19.6 | .266 | 5: 7.5 |
| 43 and 44 | .527 | .458 | 29.8 | .419 | 25.1 | .352 | 5: 7 |
| 47 and 48 | .971 | .671 | 9.7 | .352 | 15.5 | .626 | 5:14.1 |
| 55 and 56 | .315 | .2 | 34.0 | .53 | 22.8 | .397 | 5: 6 |
| 63 and 64 | 6.3 | 4.0 | 50.0 | 1.102 | 34.1 | .752 | 5: 5.7 |
| **ALFALFA HAY** | | | | | | | |
| 65 and 66 | .305 | .352 | 71.0 | 1.595 | 17.7 | .398 | 5: 2.2 |
| 67 and 68 | .527 | .458 | 78.4 | 1.56 | 35.3 | .624 | 5: 3.8 |
| 71 and 72 | .971 | .671 | 11.4 | .86 | 12.82 | .96 | 5: 9.3 |
| 79 and 80 | .315 | .2 | 85.5 | 1.684 | 25.0 | .492 | 5: 2.4 |
| 87 and 88 | 6.30 | 4.0 | 98.5 | 1.84 | 27.8 | .521 | 5: 2.3 |
| **ALFALFA ROOTS** | | | | | | | |
| 65 and 66 | .305 | .352 | 21.46 | .326 | 34.0 | .462 | 5:11.8 |
| 67 and 68 | .527 | .458 | 21.2 | .314 | 37.5 | .558 | 5:14.7 |
| 71 and 72 | .971 | .671 | 10.64 | .628 | 17.2 | 1.00 | 5:13.3 |
| 79 and 80 | .315 | .2 | 25.0 | .449 | 30.0 | .54 | 5:10 |
| 87 and 88 | 6.30 | 4.0 | 96.0 | 2.2 | 59.2 | 1.358 | 5: 5.1 |

EFFECT OF MAGNESIUM AND CALCIUM IN DOLOMITE, MAGNESITE, AND PREPARED CARBONATES UPON WHEAT AND ALFALFA IN SAND (SERIES E AND F)

Table VI shows the yields of wheat and alfalfa when grown in sand and treated with increasing amounts of magnesium in magnesite. The applications vary between 0.1 per cent of magnesium and 0.6 per cent of magnesium or 0.35 to 2.1 per cent of megnesite. Chemically pure calcium carbonate was added to make a ratio of calcium to magnesium equal to 5 to 4. The wheat and alfalfa in these two series were grown to maturity, the seeds being ground up with the straw and an analysis of the composite made.

There was but little seed upon the alfalfa, owing to the fact that it was grown under screens and the fertilization was poor. However, the treatments seemed to cause no injury except where magnesium was applied in the form of the prepared carbonates in pots 104 and 120.

TABLE VI.—*Yields of wheat and alfalfa (in grams per pot on a water-free basis) in magnesite—series E and F*

| Treatment. | Molecular ratio of calcium to magnesium. | Wheat, series E. | | Alfalfa, series F. | | | |
|---|---|---|---|---|---|---|---|
| | | Pot No. | Tops. | Pot No. | First crop, tops. | Second crop, tops. | Roots. |
| None | ..... | 89 | 7.25 | 105 | 6.86 | 5.2 | 3.78 |
| Percentage of magnesium in magnesite plus calcium carbonate: | | | | | | | |
| 0.1 | 5:4 | 90 | 6.53 | 105 | 6.00 | 5.36 | 7.13 |
| | 5:4 | 91 | 7.16 | 107 | 8.35 | 5.8 | 5.46 |
| .2 | 5:4 | 92 | 5.98 | 108 | 6.77 | 6.16 | 7.48 |
| | 5:4 | 93 | 9.88 | 109 | 6.25 | 4.94 | 4.57 |
| .3 | 5:4 | 94 | 10.96 | 110 | 5.72 | 5.2 | 3.96 |
| | 5:4 | 95 | 6.8 | 111 | 5.9 | 5.1 | 4.32 |
| .4 | 5:4 | 96 | 7.07 | 112 | 5.64 | 4.48 | 4.48 |
| | 5:4 | 97 | 7.8 | 113 | 6.77 | 5.02 | 1.85 |
| .5 | 5:4 | 98 | 9.15 | 114 | 6.07 | 4.31 | 1.49 |
| | 5:4 | 99 | 7.8 | 115 | 7.92 | 4.4 | 2.38 |
| .6 | 5:4 | 100 | 11.4 | 116 | 7.48 | 5.2 | 5.02 |
| | 5:4 | 101 | 6.25 | 117 | 7.32 | 5.55 | 3.52 |
| Percentage of magnesium in dolomite $C_3$: | | | | | | | |
| 0.6 | 5:5.2 | 102 | 9.42 | 118 | 6.34 | 5.9 | 4.93 |
| | 5:5.2 | 103 | 10.86 | 119 | 5.72 | 4.66 | 2.64 |
| Percentage of magnesium in magnesium carbonate | 5:4 | 104 | ..... | 120 | ..... | ..... | ..... |

Table VII shows that the higher treatments have higher contents of calcium and magnesium in the plants. The wheat grown in sand (pot 89) shows only 3.3 pounds of calcium and 2.6 pounds of magnesium per ton, as against the pots treated with larger quantities of magnesite

(100 and 101), which show 5.52 pounds of calcium and 10.88 pounds of magnesium.

Gile and Ageton (5, p. 44) show that many plants such as soybeans, sugar cane, and sunflower have higher lime contents when grown upon calcareous soils and that the increase in lime content tends to decrease the amount of magnesia, iron, and potash.

TABLE VII.—*Analysis of wheat and alfalfa grown in magnesite—series E and F*

WHEAT STRAW

| Pot No. | Substance added. | | Composition of plants. | | | | Molecular ratio of calcium to magnesium. |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium. | Calcium. | | Magnesium. | | |
| | *Per cent.* | *Per cent.* | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| 89 | 0.014 | 0.016 | 12.0 | 0.165 | 9.36 | 0.130 | 5: 6.5 |
| 90 and 91 | .222 | .100 | 10.55 | .154 | 16.55 | .241 | 5:13 |
| 100 and 101 | 1.332 | .60 | 24.4 | .276 | 48.1 | .544 | 5:16.4 |
| 102 and 103 | .967 | .60 | 24.1 | .237 | 32.3 | .317 | 5:11.1 |
| ALFALFA HAY | | | | | | | |
| 105 | .014 | .016 | 87.4 | 1.275 | 41.0 | .598 | 5:9.3 |
| 106 and 107 | .222 | .100 | 106.8 | 1.49 | 45.1 | .63 | 5:3.8 |
| 116 and 117 | 1.332 | .60 | 98.5 | 1.345 | 56.0 | .69 | 5:4.2 |
| 118 and 119 | .967 | .60 | 93.2 | 1.55 | 39.2 | .653 | 5:3.5 |
| ALFALFA ROOTS | | | | | | | |
| 105 | .014 | .016 | 25.4 | .672 | 14.52 | .385 | 5:4.8 |
| 106 and 107 | .222 | .10 | 55.7 | .89 | 43.8 | .64 | 5:6 |
| 116 and 117 | 1.332 | .60 | 63.0 | 1.48 | 61.1 | 1.434 | 5:8 |
| 118 and 119 | .967 | .60 | 65.2 | 1.73 | 35.8 | .95 | 5:4.6 |

EFFECT OF MAGNESIUM AND CALCIUM IN CALCAREOUS SOIL, MAGNESITE, DOLOMITE, AND PREPARED CARBONATES UPON WHEAT AND ALFALFA (SERIES G AND H)

It can be seen from Table VIII that the ratios of calcium to magnesium vary from one of 5 to 3.8 to one of 5 to 125 and that in both cases considerable growth occurred. However, in pots 135, 136, 161, and 162, receiving 35 per cent of magnesite and 100 gm. of calcium carbonate, the plants showed a yellow color and some sickness; still, in the case of alfalfa the plants were able to set some seed. The yields in pots 123, 124, 149, and 150, which received calcareous soil only, were somewhat less than where sand was mixed with the soil, owing to the soil being decidedly plastic and possessing a less desirable physical condition.

Plate LXXXIV shows the difference between some of the treatments in series G. Observe the small growth of the wheat in the pot receiving 6 per cent of magnesium in magnesite. This is due to the physical condition caused by applying the magnesite in a finely ground form, which caused a setting that resembled cement, whereas in the pot with 10 per cent of magnesium almost twice as much magnesite was applied, but in a coarser form. For the ratios in these pots see Table VIII.

Plate LXXXIV shows alfalfa growing under similar treatments.

TABLE VIII.—*Yields of wheat and alfalfa (in grams per pot on water-free basis) in soil, dolomite, and magnesite—series G and H*

| Treatment. | Molecular ratio of calcium to magnesium. | Wheat, series G. | | | Alfalfa, series H. | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pot No. | Tops. | Roots. | First crop. | Second crop. | Third crop. | Pot No. |
| None | ..... | 121 | 15.4 | 6.53 | 3.6 | 9.59 | 5.63 | 147 |
| | ..... | 122 | 1.08 | ..... | 2.28 | ..... | ..... | 148 |
| Percentage of magnesium in calcareous soil: | | | | | | | | |
| 2.64 | 5:3.8 | 123 | 21.93 | 7.15 | 7.22 | 7.47 | 12.6 | 149 |
| | 5:3.8 | 124 | 21.0 | 14.7 | 7.04 | 7.22 | 10.02 | 150 |
| 1.328 | 5:3.8 | 125 | 26.9 | 16.2 | 9.41 | 11.7 | 13.0 | 151 |
| | 5:3.8 | 126 | 27.3 | 10.4 | 9.59 | 12.35 | 10.9 | 152 |
| 0.672 | 5:3.8 | 127 | 24.5 | 19.4 | 10.11 | 13.72 | 12.3 | 153 |
| | 5:3.8 | 128 | 28.62 | 9.25 | 10.02 | 12.3 | 9.59 | 154 |
| 0.344 | 5:3.9 | 129 | 31.7 | 14.4 | 10.28 | 9.68 | 10.71 | 155 |
| | 5:3.9 | 130 | 32.6 | 10.32 | 9.68 | 10.46 | 7.83 | 156 |
| Percentage of magnesium in magnesite plus calcium carbonate: | | | | | | | | |
| 2.0 | 5:4 | 131 | 35.5 | 11.05 | 9.15 | 8.97 | 8.8 | 157 |
| | 5:4 | 132 | 35.8 | 10.05 | 10.9 | 9.86 | 8.88 | 158 |
| 6.0 | 5:75 | 133 | 9.7 | 4.26 | 5.28 | 8.45 | 5.2 | 159 |
| | 5:75 | 134 | 30.8 | 1.18 | 5.9 | 10.62 | 3.69 | 160 |
| 10.0 | 5:125 | 135 | 18.2 | 6.25 | 4.48 | 5.02 | 2.28 | 161 |
| | 5:125 | 136 | 17.4 | 6.16 | 10.11 | 9.77 | 9.59 | 162 |
| Percentage of magnesium in dolomite C1: | | | | | | | | |
| 12.7 | 5:5.2 | 137 | 2.17 | ..... | 5.2 | 9.77 | 9.77 | 163 |
| | 5:5.2 | 138 | 3.26 | ..... | ..... | ..... | ..... | 164 |

One of the most noticeable facts brought out in these series is the great sensitiveness of the plants to small quantities of calcium and magnesium, also their ability to utilize relatively insoluble forms of these two materials. In pots 121 and 122, Table IX, and pots 147 and 148, Table X, the plants were grown in extracted sand receiving no calcium and magnesium and were able to obtain considerable quantities that had not been removed by the acid extractions. The alfalfa was even able to mature a few seeds.

TABLE IX.—*Analysis of wheat grown in soil, dolomite, and magnesite—series G*

WHEAT STRAW

| Pot No. | Substance added. | | Composition of plants. | | | | |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium. | Calcium. | | Magnesium. | | Molecular ratio of calcium to magnesium. |
| | | | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| 121 | 0.014 | 0.016 | 24.65 | 0.165 | 20.4 | 0.132 | 5: 7 |
| 123 and 124 | 5.78 | 2.64 | 76.1 | .358 | 52.9 | .249 | 5: 5.7 |
| 125 and 126 | 2.897 | 1.328 | 130.0 | .48 | 78.7 | .29 | 5: 5 |
| 129 and 130 | .732 | .344 | 156.1 | .485 | 97.5 | .305 | 5: 5.2 |
| 131 and 132 | 4.44 | 2.0 | 88.6 | .248 | 188.0 | .527 | 5:17.7 |
| 135 and 136 | .666 | 10.0 | 66.8 | .375 | 170.0 | .955 | 5:21.2 |
| 137 and 138 | 20.47 | 12.7 | 15.6 | .574 | 19.9 | .73 | 5:12.7 |

WHEAT ROOTS

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 121 | .014 | .016 | 18.7 | .287 | 8.14 | .125 | 5:3.6 |
| 123 and 124 | 5.78 | 2.64 | 85.7 | .795 | 35.6 | .33 | 5:3.4 |
| 125 and 126 | 2.897 | 1.328 | 76.5 | .584 | 44.3 | .332 | 5:4.8 |
| 129 and 130 | .732 | .344 | 63.5 | .513 | 30.7 | .248 | 5:4 |
| 131 and 132 | 4.44 | 2.0 | 129.4 | 1.224 | 163.9 | 1.6 | 5:10.8 |
| 135 and 136 | .666 | 10.0 | 22.25 | .358 | 44.75 | .72 | 5:16.8 |

The high percentage of magnesium in the plants grown in pots receiving 35 per cent of magnesite is also characteristic of tolerance. Likewise a high magnesium content tends to accompany plant sickness. In the case of wheat grown in dolomite, pots 137 and 138, there was a higher percentage of calcium than in any other treatment. A ton of water-free material contained 11.48 pounds of calcium and 14.6 pounds of magnesium, but a ton of dry matter from the treatment with 25 per cent of magnesite showed 7.5 pounds of calcium and 19.1 pounds of magnesium per ton, as against the check in sand which contained 3.3 pounds of calcium and 2.64 pounds of magnesium. Alfalfa tends to show the same thing, except that it is a decidedly heavier feeder upon these two elements than is the wheat crop.

The wheat, Table VIII, was planted on January 26, 1914, and harvested on May 27, 1914, making 121 days of growth. The alfalfa was also planted on the above date and the first crop harvested on May 27, 1914. The second crop was harvested 127 days later, on October 1, 1914, and the third crop on November 12, 1914, after 42 days of additional growth.

By comparing pot 147, Table X, for the three crops, it will be seen that the second crop of alfalfa contained practically three times as much calcium and magnesium per ton as did the first crop, while the time of growth was about the same. The third crop contained about twice as much calcium and magnesium per ton as did the first crop, and its period

of growth was only 42 days. This is due chiefly to the extensive development of roots, making it possible to utilize more of the small quantities of calcium and magnesium remaining in the extracted sand, for in the other pots where these two elements were added such striking differences do not occur in the different crops.

To each pot receiving 15 alfalfa seeds, 0.19 mgm. of calcium and 0.32 mgm. of magnesium were added in the seed, and for the three crops 164.5 mgm. of calcium and 90.72 mgm. of magnesium were removed. This indicates to what extent the plants may attack relatively insoluble compounds.

TABLE X.—*Analysis of alfalfa grown in soil, dolomite, and magnesite—series H*

ALFALFA, FIRST CROP

| Pot No. | Substance added. | | Composition of plants. | | | | |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium. | Calcium. | | Magnesium. | | Molecular ratio of calcium to magnesium. |
| | | | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| 147 and 148 | 0.014 | 0.016 | 11.2 | 0.382 | 5.72 | 0.198 | 5: 4.3 |
| 149 and 150 | 5.78 | 2.64 | 98.41 | 1.382 | 26.52 | .373 | 5: 2.2 |
| 151 and 152 | 2.897 | 1.328 | 136.0 | 1.432 | 33.92 | .357 | 5: 2 |
| 155 and 156 | .732 | .344 | 127.8 | 1.28 | 34.5 | .346 | 5: 2.2 |
| 157 and 158 | 4.44 | 2.0 | 92.5 | .922 | 74.5 | .744 | 5: 6.8 |
| 159 and 160 | .666 | 6.0 | 23.8 | .426 | 52.3 | .936 | 5:18.3 |
| 161 and 162 | .666 | 10.0 | 76.7 | 1.05 | 83.7 | 1.148 | 5: 9.1 |
| 163 | 20.47 | 12.7 | 34.2 | .66 | 33.8 | .633 | 5: 8 |
| ALFALFA, SECOND CROP | | | | | | | |
| 147 | .014 | .016 | 105.3 | 1.096 | 63.8 | .665 | 5:5 |
| 149 and 150 | 5.78 | 2.64 | 117.5 | 1.615 | 31.8 | .434 | 5:2.2 |
| 151 and 152 | 2.897 | 1.328 | 172.0 | 1.402 | 50.0 | .407 | 5:2.5 |
| 155 and 156 | .732 | .344 | 114.5 | 1.138 | 50.0 | .517 | 5:3.7 |
| 157 and 158 | 4.44 | 2.0 | 103.4 | 1.098 | 67.6 | .718 | 5:5.4 |
| 159 and 160 | .666 | 6.0 | 75.0 | .785 | 76.5 | .803 | 5:8.5 |
| 161 and 162 | .666 | 10.0 | 102.0 | 1.381 | 73.6 | 1.00 | 5:6 |
| 163 | 20.47 | 12.7 | 104.4 | 1.068 | 69.4 | .711 | 5:5.5 |
| ALFALFA, THIRD CROP | | | | | | | |
| 147 | .014 | .016 | 48.0 | .854 | 21.22 | .378 | 5:3.6 |
| 149 and 150 | 5.78 | 2.64 | 110.0 | .974 | 31.6 | .291 | 5:2.4 |
| 151 and 152 | 2.897 | 1.328 | 110.2 | .922 | 32.2 | .27 | 5:1.9 |
| 155 and 152 | .732 | .344 | 78.75 | .955 | 29.62 | .32 | 5:2.8 |
| 157 and 158 | 4.44 | 2.0 | 85.0 | .962 | 40.7 | .461 | 5:4 |
| 159 and 160 | .666 | 6.0 | 40.8 | .922 | 39.8 | .783 | 5:7 |
| 161 and 162 | .666 | 10.0 | 51.6 | .87 | 54.7 | .922 | 5:9.2 |
| 163 | 20.47 | 12.7 | 96.5 | .99 | 49.0 | .503 | 5:4.2 |

## EFFECT OF MAGNESITE AND DOLOMITE UPON WHEAT AND SOYBEANS (SERIES I AND J)

Series I had wheat grown in the pots and then turned under, and wheat was then replanted in the same pots; while series J had cowpeas grown and turned under and then soybeans planted, except in pots 182 and 183, from which the cowpeas were removed before the soybeans were planted. The cowpea hay grown in pots 182 and 183 contained 0.4 per cent of calcium and 0.179 per cent of magnesium, in a ratio of 5 to 5.7, and removed from each pot 32 mgm. of calcium and 14.32 mgm. of magnesium. In 10 seeds planted there was 0.58 mgm. of calcium and 1.59 mgm. of magnesium. The above pots contained extracted sand.

Figure 1 of Plate LXXXV shows the effect of succeeding crops when grown upon extracted sand. The pot at the left marked "sand only" has had no other crop preceding it, while in the middle pot cowpeas were grown and removed, taking out some of the most readily available calcium and magnesium. From the pot at the right three crops of alfalfa were removed, taking out 164.5 mgm. of calcium and 90.72 mgm. of magnesium.

Dolomite has no detrimental effect upon the crops used throughout these experiments. However, the addition of larger quantities of magnesite—for example, 35 per cent—caused considerable yellowing of the leaves, and the plants were able to mature but few seeds. Plate LXXXV, figure 2, shows that the plants growing in dolomite have quite a number of bean pods, while in the magnesite pot none are visible and the uppermost leaves are sickly. This yellowing of the uppermost leaves while the lower ones remain green differs from true translocation and accompanies high magnesium applications. The yellow leaves have a higher magnesium content than do the healthy ones, as sickly leaves from the plants taken from pot 185 show 0.955 per cent of calcium and 1.11 per cent of magnesium, while the healthy leaves from the same plants showed 0.896 per cent of calcium and 0.88 per cent of magnesium, respectively.

Schulze and Godet (31) found more calcium in the husk and more magnesium in the seed of lupine, pine, pumpkin, castor bean, sunflower, and various nuts.

Plate LXXXVI, figure 1, shows the comparative growths of soybeans in brown silt loam and dolomite. Evidently the brown silt loam would have been improved by applications of some limestone or dolomite.

The differences of yields of duplicates in Table XI are due chiefly to the differences in the duration of growth. In the wheat, series I, pots 173, 175, 177, and 179 were harvested 65 days after planting, while their duplicates were harvested 12 days earlier. In the soybean, series J, pots 181, 184, 186, and 188 were harvested 53 days after planting, while their duplicates were permitted to mature, standing until 80 days after planting.

TABLE XI.—*Yields of wheat and soybeans (in grams per pot on the water-free basis) in dolomite, magnesite, and sand—series I and J*

| Treatment. | Molecular ratio of calcium to magnesium. | Wheat, series I. | | | Soybeans, series J. | | |
|---|---|---|---|---|---|---|---|
| | | Pot No. | Tops. | Roots. | Pot No. | Tops. | Roots. |
| None | ..... | 173 | 0.9 | 0.4 | 181 | 1.0 | 0.5 |
| | ..... | 174 | .3 | .4 | 182 | 1.2 | ..... |
| Percentage of magnesium in magnesite plus calcium carbonate: | | | | | | | |
| 2 | 5:4 | 175 | 4.8 | 2.9 | 183 | 5.6 | ..... |
| | 5:4 | 176 | 1.6 | 1.1 | 184 | 4.7 | .9 |
| 10 | 5:125 | 177 | 5.3 | 1.7 | 185 | 4.0 | ..... |
| | 5:125 | 178 | 1.8 | 1.0 | 186 | 3.2 | .8 |
| Percentage of magnesium in dolomite $C_3$: | | | | | | | |
| 12 | 5:5.2 | 179 | 5.3 | 3.2 | 187 | 6.3 | ..... |
| | 5:5.2 | 180 | 1.9 | .9 | 188 | 3.4 | .7 |

Table XII shows the analyses of wheat grown in series I.

TABLE XII.—*Analysis of straw of wheat grown in dolomite, magnesite, and sand—series I*

| Pot No. | Substance added. | | Composition of plants. | | | | |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium. | Calcium. | | Magnesium. | | Molecular ratio of calcium to magnesium. |
| | *Per cent.* | *Per cent.* | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| 174 | 0.014 | 0.016 | 1.32 | 0.44 | 0.38 | 0.127 | 5: 2.4 |
| 173 | .014 | .016 | 1.21 | .135 | 1.26 | .14 | 5: 7.1 |
| 176 | 4.44 | 2.0 | 10.88 | .68 | 13.04 | .815 | 5: 9.9 |
| 175 | 4.44 | 2.0 | 21.36 | .445 | 36.78 | .783 | 5:14.6 |
| 178 | .666 | 10.0 | 8.87 | .493 | 18.03 | 1.002 | 5:16.9 |
| 177 | .666 | 10.0 | 16.16 | .305 | 40.54 | .765 | 5:21 |
| 180 | 20.47 | 12.7 | 10.26 | .54 | 11.38 | .599 | 5: 9.2 |
| 179 | 20.47 | 12.7 | 24.11 | .455 | 22.15 | .418 | 5: 7.6 |

Comparisons of the contents of plants at different stages of growth are reported in Table XIX.

## THE EFFECT OF MAXIMUM QUANTITY OF CALCIUM AND MAGNESIUM UPON WHEAT AND SOYBEANS IN SAND (SERIES K)

Analysis of sand treated by different methods shows the hot-extracted sand to contain only slightly less calcium but considerably less magnesium than the cold-extracted sand.

Table XIX shows the analysis of wheat and soybeans grown in such sands. It can be seen that the soybeans contained only slightly more of these two elements than was in the seed, but it must be remembered that scarcely any growth occurred. However, the wheat, pots 193 to 196, contained from 12 to 22 times as much calcium and 4 times as much

magnesium as was added in the seed. Now, in pots 199 to 202, where a small amount of easily available calcium had been applied, the percentage in the plants was materially increased.

Attempts were made to grow wheat and cowpeas in paraffin, so that they would have no access to calcium and magnesium. However, this permitted but little growth, and analyses of the total plants thus grown showed their calcium and magnesium contents to be equivalent to the amount present in the seed.

TABLE XIII.—*Analysis of wheat and soybeans grown in extracted sand—series K*

| Pot No. | Treatment of sand. | Composition of plants. | | | | | Substance added in seed. | |
|---|---|---|---|---|---|---|---|---|
| | | Calcium. | | Magnesium. | | Molecular ratio of calcium to magnesium. | Calcium. | Magnesium. |
| | | *Mgm.* | *Per ct.* | *Mgm.* | *Per ct.* | | *Mgm.* | *Mgm.* |
| **SOYBEAN PLANT** | | | | | | | | |
| 189 and 190[a] | Extracted with hydrochloric acid in the cold. | 2. 15 | 0. 293 | 2. 77 | 0. 376 | 5: 10. 6 | 1. 16 | 2. 32 |
| 191 and 192 | Extracted with hydrochloric acid on steam bath. | 1. 5 | . 335 | 1. 65 | . 369 | 5: 9. 2 | 1. 16 | 2. 32 |
| **WHEAT PLANT** | | | | | | | | |
| 193 and 194 | Extracted with hydrochloric acid in the cold. | . 67 | . 22 | . 78 | . 256 | 5: 9. 7 | . 03 | . 18 |
| 195 and 196 | Extracted with hydrochloric acid on steam bath. | . 36 | . 168 | . 72 | . 337 | 5: 16. 7 | . 03 | . 18 |
| 197 and 198 | 0.2 gm. sodium bicarbonate ($NaHCO_3$). | . 80 | . 155 | . 74 | . 144 | 5: 8 | . 03 | . 18 |
| 199 and 200 | 0.2 gm. sodium bicarbonate ($NaHCO_3$) 0.05 gm. calcium nitrate ($Ca(NO_3)_2$). | 1. 14 | . 3 | . 35 | . 093 | 5: 2. 5 | . 03 | . 18 |
| 201 and 202 | 0.2 gm. sodium bicarbonate ($NaHCO_3$) 0.05 gm. calcium nitrate ($Ca(NO_3)_2$) 0.0317 gm. magnesium sulphate ($MgSO_4$) | . 34 | . 247 | . 48 | . 35 | 5: 11. 8 | . 03 | . 18 |

[a] The containers in this series were tall Jena beakers holding 1,350 gm. of sand.

From Table XIII it can be seen that the plants contained more calcium and magnesium than was added in the seed, thus showing their power to obtain these two elements from sand that had been previously extracted with acid.

## EFFECT OF MAGNESIUM AND CALCIUM IN SULPHATES, CHLORIDS, AND CARBONATES UPON WHEAT AND SOYBEANS IN SAND (SERIES L AND M)

When calcium and magnesium were applied in sulphates, chlorids, and carbonates the smaller applications gave the highest yields. As recorded in Table XIV, 0.1 per cent of magnesium in the carbonate inhibited germination and permitted no growth, whereas this quantity in the sulphates and chlorids gave considerable growth; however, the chlorids were more detrimental than the sulphates, while at lower concentrations, such as 0.01 and 0.001 per cent of magnesium, the carbonates gave the best growth, the chlorids being the most detrimental. In the case of soybeans all the chlorid treatments permitted practically no seed formation, while treatment with smaller quantities of carbonates gave considerable seed. The root formation was relatively the same as the top growth, the detrimental effect accompanied short thick roots which appeared brownish or reddish brown. Plate LXXXVII shows this comparative root growth. Plate LXXXVIII, figure 1, shows the comparisons of wheat when grown in extracted sand and in dolomite. Figure 2 shows the retarded growth of wheat due to the chlorids of magnesium.

TABLE XIV.—*Yields of wheat and soybeans (in grams per pot on the water-free basis) in the sulphates, chlorids, and carbonates of magnesium and calcium—series L and M*

| Treatment. | Molecular ratio of calcium to magnesium. | Wheat, series L. | | | Soybeans, series M. | | |
|---|---|---|---|---|---|---|---|
| | | Pot No. | Tops. | Roots. | Pot No. | Tops. | Roots. |
| None | ..... | 219 | 5.8 | 3.0 | 237 | 1.3 | 0.3 |
| None | ..... | 220 | 2.1 | 1.0 | 238 | 2.0 | ..... |
| Percentage of magnesium in magnesium sulphate plus calcium sulphate: | | | | | | | |
| 0.1 | 5:4 | 203 | 2.4 | 1.4 | 221 | 3.2 | ..... |
| | 5:4 | 204 | 3.3 | 1.1 | 222 | 2.6 | .6 |
| .01 | 5:4 | 205 | 3.8 | 1.5 | 223 | 5.5 | ..... |
| | 5:4 | 206 | 4.4 | 1.3 | 224 | 4.9 | 1.0 |
| .001 | 5:4 | 207 | 5.1 | 1.7 | 225 | 3.6 | .7 |
| | 5:4 | 208 | 3.8 | 1.6 | 226 | 4.0 | ..... |
| Percentage of magnesium in magnesium chlorid plus calcium chlorid: | | | | | | | |
| 0.1 | 5:4 | 209 | 1.6 | .7 | 227 | 2.4 | ..... |
| | 5:4 | 210 | 1.5 | .6 | 228 | 2.1 | .6 |
| .01 | 5:4 | 211 | 3.1 | 1.3 | 229 | 2.6 | .0 |
| | 5:4 | 212 | 3.5 | 1.6 | 230 | 2.7 | .6 |
| .001 | 5:4 | 213 | 5.2 | 2.5 | 231 | 3.6 | 1.0 |
| | 5:4 | 214 | 8.8 | 4.7 | 232 | 3.5 | ..... |
| Percentage of magnesium in magnesium carbonate plus calcium carbonate: | | | | | | | |
| 0.01 | 5:4 | 215 | 6.8 | 4.0 | 233 | 2.9 | .7 |
| | 5:4 | 216 | 3.3 | 1.5 | 234 | 7.8 | ..... |
| .001 | 5:4 | 217 | 6.8 | 7.8 | 235 | 6.3 | 1.2 |
| | 5:4 | 218 | 4.2 | 2.6 | 236 | 7.6 | ..... |

In Table XIV pots 205, 208, 210, 212, 213, 216, 218, and 220 were harvested 53 days after planting. Their duplicates were permitted to grow 12 days longer. Pots 221, 223, 226, 227, 229, 232, 235, and 237 were harvested at maturity, 80 days from the time of planting. Duplicates were grown only 53 days. The analyses are given in Tables XV and XVI.

TABLE XV.—*Analysis of straw of wheat grown with sulphates, chlorids, and carbonates of calcium and magnesium—series L*

| Pot No. | Substance added. | | Composition of plants. | | | | Molecular ratio of calcium to magnesium. |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium. | Calcium. | | Magnesium. | | |
| | | | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| **SULPHATES** | | | | | | | |
| 203 | 0.222 | 0.1 | 18.48 | 0.77 | 15.45 | 0.644 | 5: 6.9 |
| 205 | .022 | .01 | 26.6 | .70 | 38.86 | .97 | 5:11.5 |
| 208 | .002 | .001 | 15.2 | .40 | 5.73 | .151 | 5: 3.1 |
| **CHLORIDS** | | | | | | | |
| 210 | .222 | .1 | 22.5 | 1.5 | 11.77 | .785 | 5: 4.3 |
| 212 | .022 | .01 | 12.67 | .362 | 9.66 | .276 | 5: 6.8 |
| 213 | .002 | .001 | 22.36 | .43 | 9.05 | .174 | 5: 3.3 |
| **CARBONATES** | | | | | | | |
| 216 | .022 | .01 | 9.24 | .28 | 20.33 | .616 | 5:18.3 |
| 218 | .002 | .001 | 17.28 | .432 | 15.68 | .392 | 5: 7.5 |
| 220 | ..... | ..... | 5.31 | .253 | 2.58 | .123 | 5: 4 |

The plants used in the experiments in Table XV were harvested when 53 days old.

TABLE XVI.—*Analysis of soybean hay grown with sulphates, chlorids, and carbonates of calcium and magnesium—series M*

| Pot No. | Substance added. | | Composition of plants. | | | | Molecular ratio of calcium to magnesium. |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium. | Calcium. | | Magnesium. | | |
| | | | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| **SULPHATES** | | | | | | | |
| 222 | 0.222 | 0.1 | 30.94 | 1.19 | 31.46 | 1.21 | 5:8.4 |
| 224 | .022 | .01 | 41.89 | .885 | 31.45 | .642 | 5:6.2 |
| 225 | .002 | .001 | 21.24 | .59 | 12.24 | .34 | 5:4.8 |
| **CHLORIDS** | | | | | | | |
| 228 | .222 | .1 | 15.39 | .733 | 13.96 | .665 | 5:7.5 |
| 230 | .022 | .01 | 25.11 | .93 | 19.54 | .724 | 5:6.4 |
| 231 | .002 | .001 | 13.89 | .386 | 10.26 | .285 | 5:6.1 |
| **CARBONATES** | | | | | | | |
| 233 | .022 | .01 | 27.26 | .94 | 23.92 | .825 | 5:7.3 |
| 235 | .002 | .001 | 72.13 | 1.145 | 38.36 | .609 | 5:4.4 |
| 237 | ..... | ..... | 7.15 | .55 | 4.04 | .311 | 5:4.7 |

EFFECT OF MAGNESIUM AND CALCIUM IN CALCAREOUS SOIL, DOLOMITE, AND MAGNESITE, AFTER ALFALFA, UPON SOYBEANS (SERIES N)

The soybeans in series N were grown after three crops of alfalfa had been removed and the roots turned under. Pots 239 and 240 showed but a small amount of growth. Pots 251, 252, and 253 showed considerable organic growth, but the plants were sickly and did not yield much seed. The yields are reported in Table XVII. Analyses of the plants, Table XVIII, show treatments with the largest quantities of magnesium in magnesite, giving the plants with the greatest magnesium content and containing as much as 29.92 pounds of magnesium per ton. Also proportionately the highest amount of calcium and magnesium were found in these pots. The check pots, 239 and 240, showed the lowest percentage of calcium and magnesium in the plants grown.

TABLE XVII.—*Yields of soybeans (in grams per pot on the water-free basis) grown after alfalfa in soil, magnesite, and sand—series N*

| Treatment. | Molecular ratio of calcium to magnesium. | Soybeans, series N. | | | |
|---|---|---|---|---|---|
| | | Pot No. | Tops. | Roots. | Seeds. |
| None | ..... | 239 | 1.3 | ..... | ..... |
| | ..... | 240 | 1.6 | ..... | ..... |
| Percentage of magnesium in calcareous soil: | | | | | |
| 2.64 | 5:3.8 | 241 | 5.6 | 0.6 | ..... |
| | 5:3.8 | 242 | 7.3 | ..... | 2.51 |
| 1.328 | 5:3.8 | 243 | 7.2 | 1.2 | ..... |
| | 5:3.8 | 244 | 6.2 | ..... | 2.2 |
| .672 | 5:3.8 | 245 | 8.5 | ..... | 4.59 |
| | 5:3.8 | 246 | 6.1 | 1.2 | ..... |
| .344 | 5:3.9 | 247 | 12.1 | ..... | 6.95 |
| | 5:3.9 | 248 | 6.5 | 1.0 | ..... |
| Percentage of magnesium in magnesite plus calcium carbonate: | | | | | |
| 0.2 | 5:4 | 249 | 6.6 | ..... | 1.84 |
| | 5:4 | 250 | 6.6 | 2.0 | ..... |
| 10 | 5:125 | 252 | 3.8 | .7 | ..... |
| | 5:125 | 253 | 8.5 | ..... | ..... |
| Percentage of magnesium in magnesite: | | | | | |
| 0.6 | 5:75 | 251 | 4.0 | .7 | ..... |
| Percentage of magnesium in dolomite $C_3$: | | | | | |
| 12.7 | 5:5.2 | 254 | 3.8 | .7 | ..... |

Pots 240, 242, 244, 245, 247, 249, and 253 were harvested at maturity, or 80 days after planting.

The plants used in the experiments in Table XVIII were 53 days old.

Table XIX shows the differences in composition of wheat grown under the same treatment but harvested at different periods of growth. The first plants were harvested 53 days after being planted. It was the original plan to allow the duplicates to mature, but owing to attacks of mildew they were harvested 12 days later.

TABLE XVIII.—*Analysis of soybeans grown after alfalfa in soil, magnesite, and sand—series N*

| Pot No. | Substance added. | | Composition of plants. | | | | Molecular ratio of calcium to magnesium. |
|---|---|---|---|---|---|---|---|
| | Calcium. | Magnesium | Calcium. | | Magnesium. | | |
| | *Per cent.* | *Per cent.* | *Mgm.* | *Per cent.* | *Mgm.* | *Per cent.* | |
| 239 | 0.014 | 0.016 | 7.02 | 0.54 | 4.6 | 0.354 | 5: 5.4 |
| 241 | 5.78 | 2.64 | 109.76 | 1.96 | 41.5 | .741 | 5: 3.1 |
| 243 | 2.897 | 1.328 | 131.0 | 1.82 | 52.7 | .732 | 5: 3.3 |
| 246 | 1.455 | .672 | 90.28 | 1.48 | 47.88 | .785 | 5: 4.4 |
| 248 | .732 | .344 | 76.62 | 1.225 | 48.42 | .745 | 5: 5.1 |
| 250 | 4.44 | 2.0 | 82.83 | 1.225 | 76.54 | 1.19 | 5: 7.9 |
| 251 | .666 | 6.0 | 29.04 | .726 | 39.40 | .985 | 5:11.3 |
| 252 | .666 | 10.0 | 32.83 | .864 | 56.85 | 1.496 | 5:14.4 |
| 254 | 20.47 | 12.7 | 46.96 | 1.236 | 31.73 | .835 | 5: 5.6 |

The percentages of calcium and magnesium were greater in the plants harvested in the earlier stages of growth. In the wheat the proportion of magnesium to calcium was somewhat greater in the later stages of growth. Still it must be remembered that the plants were by no means thoroughly matured. This was not the case with the soybeans, as is shown by Table XX. Soybean plants at maturity, or 80 days after planting, showed higher percentages of calcium and magnesium than at the end of 53 days of growth, except the checks in sand and those having had extremely small applications.

TABLE XIX.—*Composition of wheat at different stages of growth*

| Treatment. | Wheat 53 days old. | | | | Wheat 65 days old. | | |
|---|---|---|---|---|---|---|---|
| | Pot No. | Calcium. | Magnesium. | Molecular ratio of calcium to magnesium. | Calcium. | Magnesium. | Molecular ratio of calcium to magnesium. |
| None | 173 | 0.44 | 0.127 | 5: 2.4 | 0.135 | 0.14 | 5: 7.1 |
| Percentage of magnesium in dolomite $C_3$: | | | | | | | |
| 12.7 | 179 | .54 | .599 | 5: 9.2 | .455 | .418 | 5: 7.6 |
| Percentage of magnesium in magnesite: | | | | | | | |
| 10 | 177 | .493 | 1.002 | 5:16.9 | .305 | .765 | 5:21 |
| 2 | 175 | .68 | .815 | 5: 9.9 | .445 | .783 | 5:14.6 |
| Percentage of magnesium in magnesium sulphate: | | | | | | | |
| 0.1 | 203 | .77 | .644 | 5: 6.9 | .61 | .54 | 5: 7.3 |
| 0.01 | 205 | .70 | .97 | 5:11.5 | .40 | .646 | 5:13.4 |
| 0.001 | 207 | .40 | .151 | 5: 3.1 | .255 | .158 | 5: 5.1 |
| Percentage of magnesium in magnesium chlorid: | | | | | | | |
| 0.1 | 209 | 1.5 | .785 | 5: 4.3 | .984 | .604 | 5: 5 |
| 0.01 | 211 | .362 | .276 | 5: 6.8 | .30 | .31 | 5: 8.6 |
| 0.001 | 213 | .43 | .174 | 5: 3.3 | .245 | .163 | 5: 5.5 |
| Percentage of magnesium in magnesium carbonate: | | | | | | | |
| 0.01 | 215 | .28 | .616 | 5:18.3 | .405 | .587 | 5:12 |
| 0.001 | 217 | .432 | .392 | 5: 7.5 | .455 | .316 | 5: 5.8 |
| None | 219 | .253 | .123 | 5: 4 | .15 | .16 | 5: 8.8 |

Seissl (32) experimented with a large number of plants in various stages of growth and found a slight fluctuation in the ratio of calcium to magnesium in the ash analyzed in the different years. In nearly every instance there was a progressive increase in the ratio of the lime to the magnesia content towards autumn. In only two cases was the lime content greater than that of the magnesia.

TABLE XX.—*Composition of soybeans at different periods of growth*

| Treatment. | Pot No. | Soybeans 53 days old. | | | Soybeans 80 days old. | | |
|---|---|---|---|---|---|---|---|
| | | Calcium. | Magnesium. | Molecular ratio of calcium to magnesium. | Calcium. | Magnesium. | Molecular ratio of calcium to magnesium. |
| | | *Per cent.* | *Per cent.* | | *Per cent.* | *Per cent.* | |
| None | 181 | 0.344 | 0.449 | 5:10.8 | 0.29 | 0.28 | 5: 8 |
| Percentage of magnesium in dolomite $C_3$: | | | | | | | |
| 12.7 | 187 | 1.546 | .964 | 5: 5.2 | 2.07 | 1.3 | 5: 5.2 |
| Percentage of magnesium in magnesite: | | | | | | | |
| 10 | 185 | .96 | 1.032 | 5: 9 | .75 | 1.138 | 5:12.6 |
| 2 | 183 | 1.357 | .838 | 5: 5.2 | 1.15 | .815 | 5: 5.9 |
| Percentage of magnesium in magnesium sulphate: | | | | | | | |
| 0.1 | 221 | 1.19 | 1.21 | 5: 8.4 | 1.71 | 2.28 | 5:11.1 |
| .01 | 223 | .855 | .642 | 5: 6.2 | 1.215 | 1.31 | 5: 9 |
| .001 | 225 | .59 | .34 | 5: 4.8 | .6 | .356 | 5: 4.8 |
| Percentage of magnesium in magnesium chlorid: | | | | | | | |
| 0.1 | 227 | .733 | .665 | 5: 7.5 | 2.55 | 1.33 | 5: 4.3 |
| .01 | 229 | .93 | .724 | 5: 6.4 | .65 | .495 | 5: 6.3 |
| .001 | 231 | .386 | .285 | 5: 6.1 | .27 | .255 | 5: 7.9 |
| Percentage of magnesium in magnesium carbonate: | | | | | | | |
| 0.01 | 233 | .94 | .825 | 5: 7.3 | 1.13 | 1.19 | 5: 8.7 |
| .001 | 235 | 1.145 | .609 | 5: 4.4 | 1.57 | .399 | 5: 2.1 |
| None | 237 | .55 | .311 | 5: 4.7 | .36 | .184 | 5: 4.2 |
| Percentage of magnesium in calcareous soil: | | | | | | | |
| 2.64 | 241 | 1.96 | .741 | 5: 3.1 | 3.0 | .997 | 5: 2.77 |
| 1.32 | 243 | 1.82 | .737 | 5: 3.3 | 3.65 | 1.26 | 5: 2.87 |
| .672 | 245 | 1.48 | .785 | 5: 4.4 | 2.7 | 1.016 | 5: 3.1 |
| .344 | 247 | 1.225 | .745 | 5: 5.1 | 2.11 | .79 | 5: 3.1 |
| None | 239 | .54 | .354 | 5: 5.4 | .17 | .118 | 5: 9.2 |
| Percentage of magnesium in magnesite: | | | | | | | |
| 2 | 249 | 1.255 | 1.19 | 5: 7.9 | 1.55 | 1.55 | 5: 8.3 |
| 10 | 252 | .864 | 1.496 | 5:14.4 | 1.145 | 2.166 | 5:15.75 |

TABLE XXI.—*Tolerance of crops for calcium and magnesium*

| Treatment. | Molecular ratio of calcium to magnisium. | Wheat. | | | Alfalfa. | | |
|---|---|---|---|---|---|---|---|
| | | Calcium. | Magnesium. | Molecular ratio of calcium to magnesium. | Calcium | Magnesium. | Molecular ratio of calcium to magnesium. |
| None | 5:9.5 | 0.187 | 0.108 | 5: 5.1 | 0.347 | 0.164 | 5:3.9 |
| Percentage of magnesium in dolomite C1: | | | | | | | |
| 0.2 | 5:4.8 | .298 | .271 | 5: 7.6 | 2.565 | .431 | 5:1.4 |
| 0.6 | 5:4.8 | .386 | .421 | 5: 9.1 | 2.622 | .649 | 5:2 |
| Percentage of magnesium in brown-gray silt loam: | | | | | | | |
| 0.352 | 5:9.6 | .296 | .256 | 5: 7.2 | 1.595 | .398 | 5:2.2 |
| Percentage of magnesium in dolomite C3: | | | | | | | |
| 12.7 | 5:5.2 | .574 | .730 | 5:12.6 | 1.068 | .711 | 5:5.5 |
| Percentage of magnesium in magnesite: | | | | | | | |
| 10 | 5:125 | .375 | .955 | 5:21 | 1.381 | 1.00 | 5:6 |

The yields in the pots in Table XXI were practically the same on the different treatments. This shows the alfalfa to contain more calcium and magnesium than the wheat. Some of the other treatments show higher percentages of calcium and magnesium, but the yields are not comparable. It might be interesting to note that soybean hay at maturity contained per ton as much as 73 pounds of calcium and 25.2 pounds of magnesium when grown in a mixture of equal parts of sand and calcareous soil, but when grown in a mixture containing 40 per cent of magnesite there were 22.9 pounds of calcium and 43.3 pounds of magnesium per ton.

## DISCUSSION

The experiments reported here extend over a period of three years (1912 to 1915) and include approximately 300 pot cultures and upwards of 300 duplicate determinations of calcium and magnesium.

Difficulty was experienced in finding a medium that was free from calcium and magnesium, and which would still approach soil conditions. Attempts were made to grow plants in aluminum turnings but without success, probably due to the formation of some aluminum salts when the plant foods were added. It is well known that aluminum salts disturb the physiological functioning of plant organs.

Wheat and cowpeas grown in granular paraffin without the addition of calcium and magnesium showed in the total plant only an amount equal to that furnished by the seed.

The difference in the medium in which the plants were grown caused different effects upon the plants. Brown silt loam was a better medium than sand when treated with chemically pure magnesium carbonate, even though it already contained 25 times as much calcium and magnesium as did the sand. Still sand would have an ameliorating effect when compared with solution cultures. Jensen (10) found that in quartz sand a much higher concentration of salts was required to cause death than in water cultures.

As previously shown under literature studies it is quite generally believed that plants have to some extent a selective absorption. The results here seem to indicate such a condition, for the dolomites used tend to go into solution in a molecular ratio, but the plants failed to take them up in this ratio. The tendency of the plants under these conditions was to take up relatively larger molecular proportions of magnesium than of calcium. Analysis of the plants show that they do not necessarily take up calcium and magnesium in the same ratio as applied, as, for example, in dolomite $C_3$ the ratio of calcium to magnesium is 5:5.2, while the plants may and do take it up in a ratio of 5:7 or 5:3.95.

In the case of the addition of 25 per cent of magnesite the ratio of calcium to magnesium was 5:125, while in some of the plants grown in such treatment the ratio varied from 5:15 to 5:21. Wheat grown in soil treated with 6 per cent of dolomite showed in the tops a ratio of 5:9.1 and in the roots a ratio of 5:4.35, or for the whole plant a ratio of 5:6.3, while in dolomite $C_1$ it was 5:4.8. Alfalfa grown in the same treatment showed for the entire plant a ratio of 5:4.2, but when grown in soil treated with dolomite $C_3$ the ratio for the total alfalfa plant was 5:3.95, while in the dolomite the ratio of the calcium to the magnesium was 5:5.2.

The chlorids of calcium and magnesium were more detrimental to wheat and soybeans than were the sulphates at concentrations up to 0.1 per cent of magnesium. This amount of magnesium in the prepared carbonate entirely inhibited growth, whereas lower concentration gave better growth than either the sulphates or chlorids.

Wheat 65 days old showed smaller percentages of calcium and magnesium than did similarly treated wheat at 53 days of growth, but the total amount of these two elements in the plants increased with the duration of growth.

Soybeans at maturity, or 80 days after planting, showed for the hay higher calcium and magnesium contents than at 53 days of growth, except in the case of the checks and those treated with extremely small quantities. Some of the samples showed as much as 73 pounds of calcium and 25.2 pounds of magnesium per ton when grown in a mixture of one-half sand and one-half calcareous soil, but when grown in soil containing 35 per cent of magnesite there were 22.9 pounds of calcium

and 42.3 pounds of magnesium per ton; whereas the checks contained 5.8 pounds of calcium and 5.6 pounds of magnesium.

Whenever excessive amounts of magnesium were applied, there was a characteristic appearance of yellow leaves. The uppermost leaves became yellow and gradually died, while the lower leaves remained green. This condition is characteristic of magnesium sickness and just the reverse of the effects produced by translocation processes.

The general tendency is for the percentages of calcium and magnesium in the plants to increase with the increase in size of application. Likewise a high magnesium content in the plant tends to accompany plant sickness, as sickly and healthy leaves from the same soybean plant showed, respectively, 1.11 per cent of magnesium as against 0.88 per cent magnesium.

All varieties of the seed used contained more magnesium than calcium, while ordinarily the remainder of the plant contained more calcium than magnesium. This conforms with the data of Schulze and Godet, who report more calcium in the husk and more magnesium in the seed.

Nitrogen was applied to the legumes as well as to the cereals, so as to be sure that this was not the limiting factor.

In a number of instances the differences in the yields between duplicates were as great as between the different treatments. At several periods during the growth of the plants parasites caused injuries, sometimes great enough to necessitate harvesting the crop.

## CONCLUSIONS

(1) Wheat, soybeans, alfalfa, and cowpeas grew normally either in 96 per cent of dolomite and 4 per cent sand, 100 per cent of magnesian limestone, or in sand containing 7 per cent of magnesite.

(2) Dolomite up to 40 per cent proved beneficial to plant growth. These results indicate that dolomite and magnesian limestone will not be detrimental as applied in agricultural practices.

(3) Applications of prepared magnesium carbonate up to 0.7 per cent caused no injury in brown silt loam, but 0.35 per cent prevented the growth of all plants tested in sand.

(4) The crop yields and the ratio of calcium to magnesium in the plants bear no direct relation to the ratio in the natural carbonates applied.

(5) Different ratios of calcium to magnesium within rather wide limits produced no marked differences in yields.

(6) Increasing the size of applications increased the calcium and magnesium content of plants.

(7) A tolerance of calcium and magnesium occurred in all varieties of plants grown. With approximately identical yields, wheat straw

grown in sand, brown silt loam, dolomite, and soil containing 35 per cent of magnesite showed calcium contents varying between 0.165 per cent and 0.547 per cent and magnesium contents varying between 0.132 per cent and 0.955 per cent.

(8) Acid extractions failed to remove all the calcium and magnesium from the sand. There remained after the various extractions from 768 to 852 mgm. of calcium and from 540 to 960 mgm. of magnesium per 6,000 gm. of sand.

(9) The plants possessed a decided ability to obtain calcium and magnesium from sand extracted with strong hydrochloric acid, as borne out by the following example: Three crops of alfalfa removed from acid extracted sand 164.43 mgm. more calcium and 90.4 mgm. more magnesium than was contained in seeds similar to those planted.

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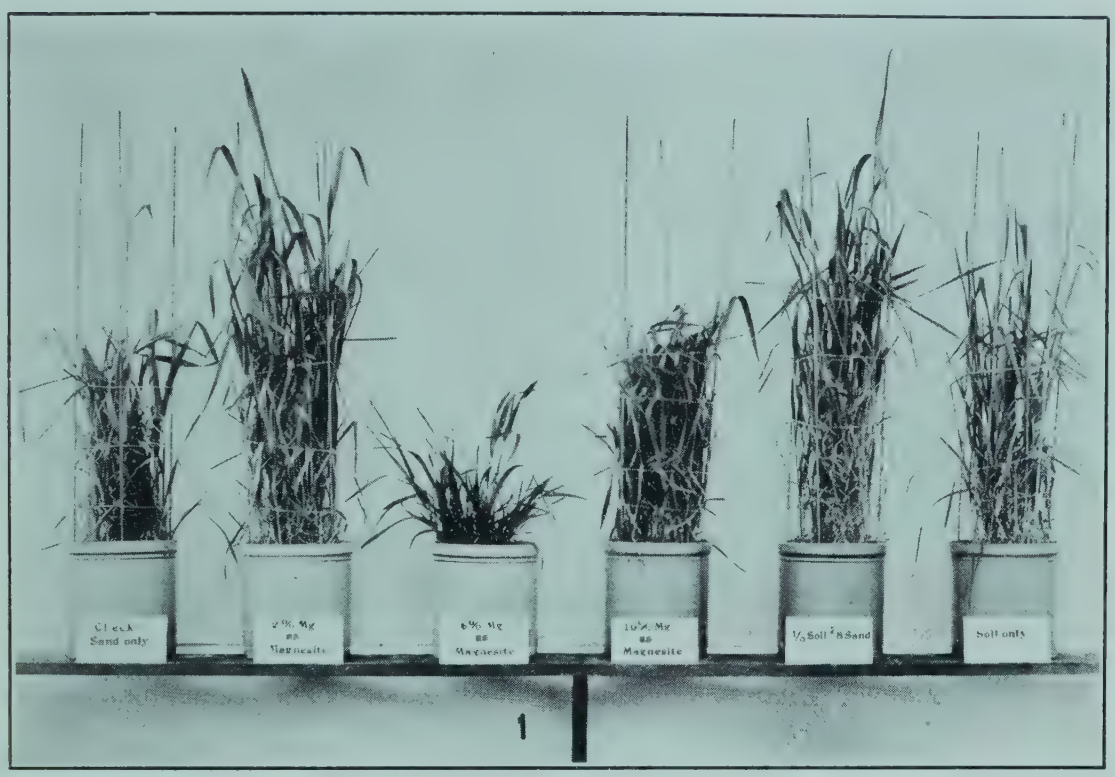
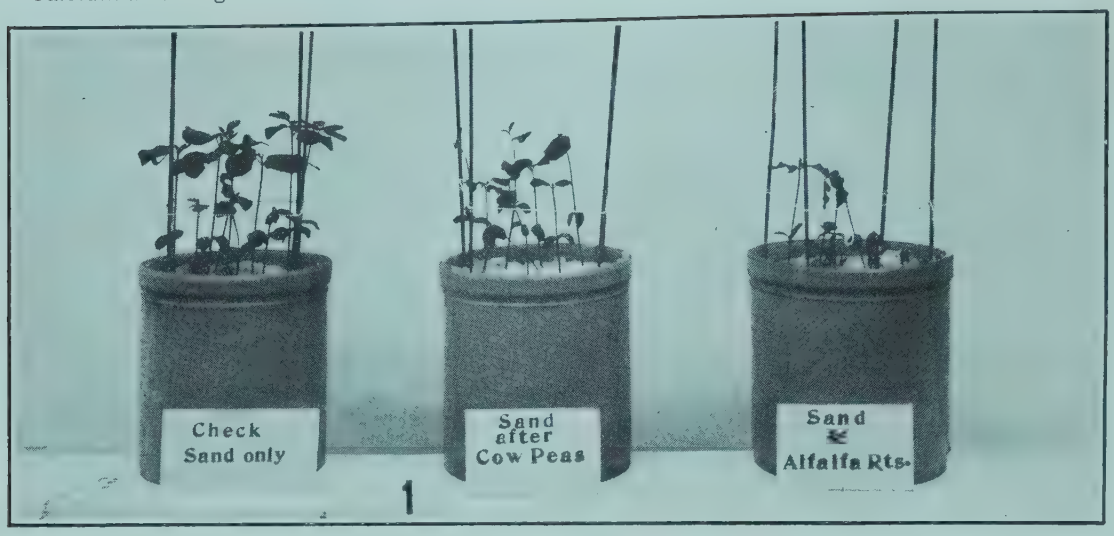
## PLATE LXXXIV

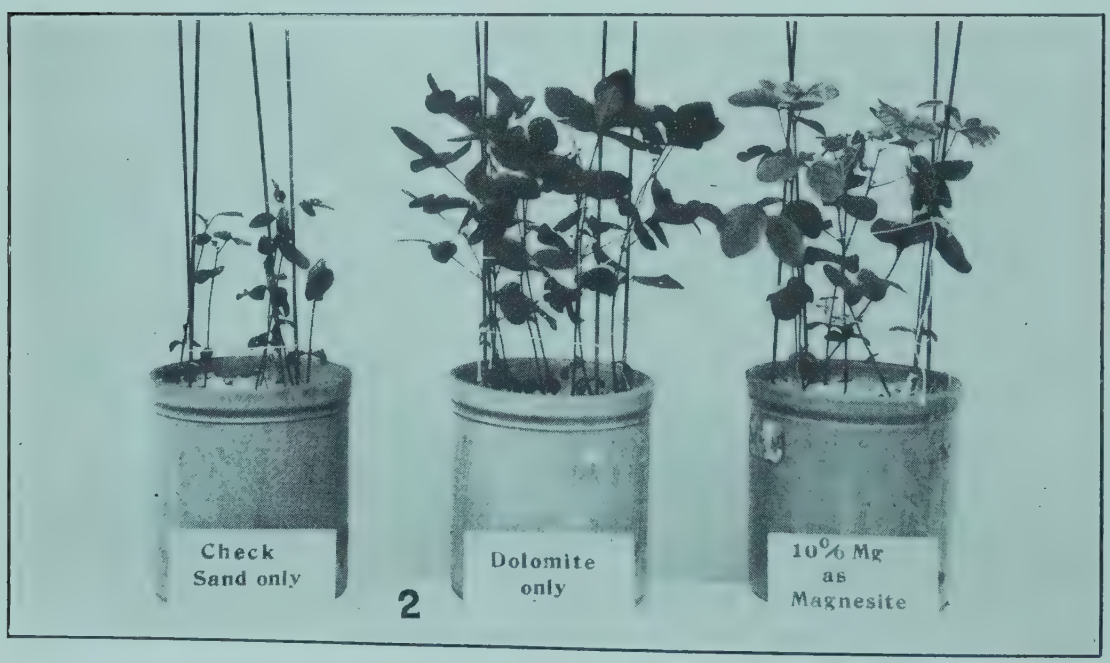
Fig. 1.—Growth of wheat in sand containing varying quantities of calcium and magnesium. The small growth of wheat in the pot marked "6% magnesium" is due to a detrimental physical effect.

Fig. 2.—Growth of alfalfa in sand containing varying amounts of calcium and magnesium.


Check
Sand only
Magnesite
Magnesite
Magnesite
1/3 Soil 2/3 Sand
Soil only
1

Check
Sand only
Magnesite
Magnesite
Magnesite
Dolomite
only
1/3 Soil 2/3 Sand
Soil only
2


Check
Sand only
Sand
after
Cow Peas
Sand
&
Alfalfa Rts.
1


Check
Sand only
Dolomite
only
10% Mg
as
Magnesite
2

## PLATE LXXXV

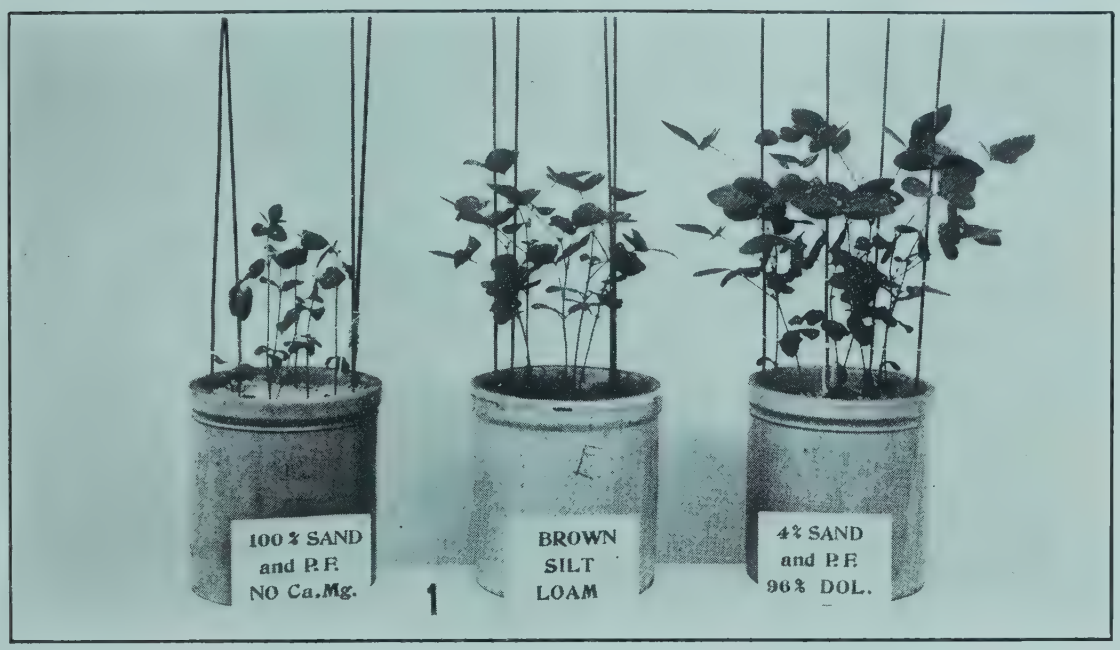
Fig. 1.—Growth of soybeans following a crop which had already absorbed most of the readily available calcium and magnesium.

Fig. 2.—Growth of soybeans in soil treated with magnesium. Note the sickly appearance of the top leaves in the right-hand pot, which is characteristic of treatment with large quantities of magnesium.

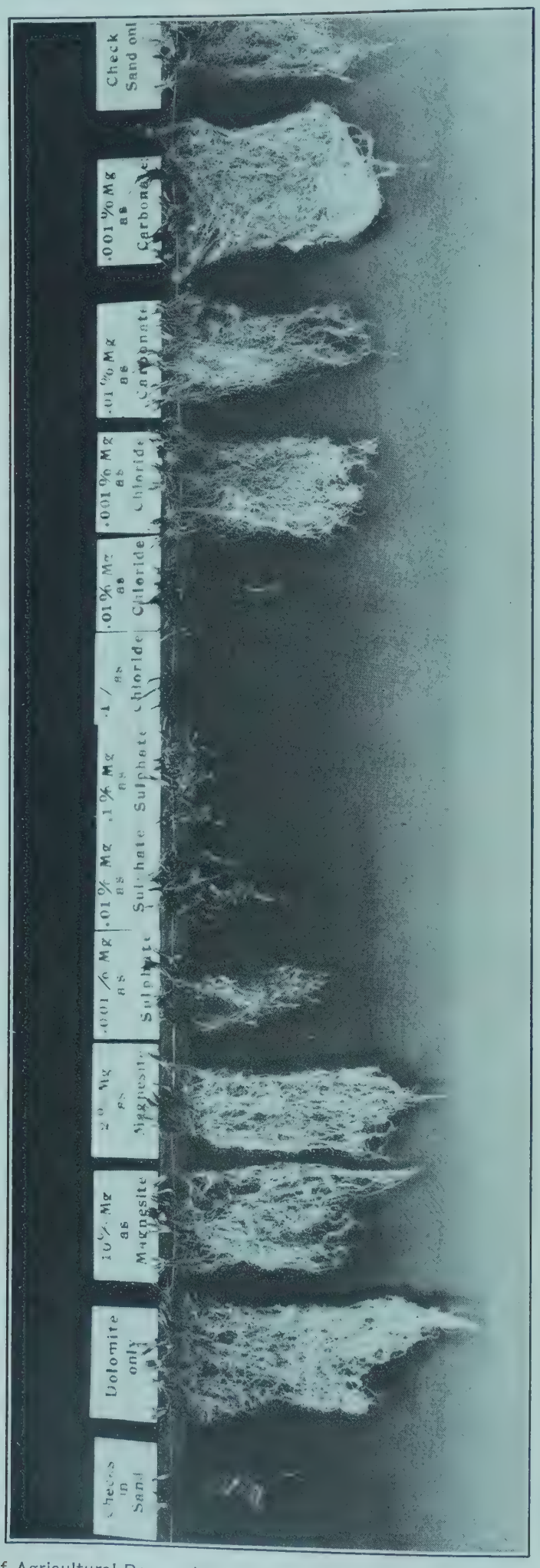
## PLATE LXXXVI

Fig. 1.—Comparative growth of soybeans in brown silt loam and dolomite, showing that the loam would have been improved by the addition of some limestone or dolomite.

Fig. 2.—Soybeans in sand treated with magnesium, showing that their growth increases inversely with the quantity of magnesium applied as sulphate.


100% SAND
and P.F.
NO Ca.Mg.
BROWN
SILT
LOAM
4% SAND
and P.F.
96% DOL.
1

Check
Sand only
.1% Mg
as
Sulphate
.01% Mg
as
Sulphate
.001% Mg
as
Sulphate
2


Check
Sand only
.001% Mg
as
Carbonate
.01% Mg
as
Carbonate
.001% Mg
as
Chloride
.01% Mg
as
Chloride
.1 /
as
Chloride
.1% Mg
as
Sulphate
.01% Mg
as
Sulphate
.001% Mg
as
Sulphate
Dolomite
only

## PLATE LXXXVII

Comparative root production of wheat grown in the chlorids, sulphates, and carbonates of magnesium and calcium. Root growth is inversely proportional to the amount of salt applied.

## PLATE LXXXVIII

Fig. 1.—Comparative growth of wheat in sand and in dolomite. The dolomite contained 96 per cent of carbonates of calcium and magnesium and 4 per cent of insoluble silica residues.

Fig. 2.—Comparative growth of wheat in magnesium chlorid and magnesium sulphate.

1
100% SAND
and P.F.
NO Ca.Mg.
4% SAND
and P.F.
96% DOL.

2
.1% Mg
as
Chloride
.1% Mg
as
Sulphate

# LARVAL CHARACTERS AND DISTRIBUTION OF TWO SPECIES OF DIATRAEA

By T. E. Holloway,[1]

*Entomological Assistant, Southern Field Crop Insect Investigations, Bureau of Entomology*

In May, 1911, Dyar[2] published an article differentiating the American species of Diatraea. The species *D. saccharalis*, commonly known as the sugar-cane moth borer and also as the larger corn stalk borer, which had been recorded in the literature as infesting corn and sugar cane throughout the Southern States, was divided into *D. saccharalis crambidoides* Fabricius and *D. zeacolella* Dyar. This paper came as a surprise to entomologists; and while in it Dyar scarcely mentions food plants it was assumed that *D. zeacolella* was supposed to infest corn (*Zea mays*) and not sugar cane (*Saccharum officinarum*), while *D. saccharalis crambidoides* was supposed to breed in sugar cane. The foundation for this belief, aside from the use of the term "zeacolella" for one of the forms, is evidently a statement made by Dyar before the Entomological Society of Washington in 1911,[3] which is noted in the Proceedings as follows:

> Dr. Dyar spoke of the troublesome genus Diatraea and announced his success in separating as two distinct species the forms feeding on corn and sugar cane in the United States in characters of both the larvæ and the adults.

After the publication of Dyar's article, series of specimens were reared from corn and sugar cane at the laboratory of Sugar-Cane Insect Investigations at Audubon Park, New Orleans, La., and these were found by Mr. U. C. Loftin to interbreed. Specimens from both sugar cane and corn were determined by Dr. Dyar as *D. saccharalis crambidoides*.

Within the last two or three years, in the field investigations of sugar-cane insects, *D. saccharalis crambidoides* has been found to be limited to the southern half of Louisiana, including the southwestern corner of Mississippi around Woodville, to the southern half of Florida, and to the lower Rio Grande Valley in Texas. There was no doubt, however, about the correctness of the records of species of Diatraea from Virginia and the Carolinas, and the writer was at a loss to explain the divergence between his records and the statements in economic literature

[1] The writer gratefully acknowledges the assistance of Mr. August Busck and Rev. J. J. De Gryse, who very kindly criticized the drawings and descriptions and helped in many ways.

[2] Dyar, H. G. The American species of Diatraea Guilding (Lepid., Pyralidæ). *In* Ent. News, v. 22, no. 5, p. 199–206. 1911.

[3] Proc. Ent. Soc. Wash., v. 13, no. 2, p. 87. 1911.

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that *D. saccharalis* occurs throughout the Southern States. Referring to Dyar's paper, he noticed that the range of *D. saccharalis crambidoides* is given as "Mexico, numerous localities, Gulf States, and lower Mississippi Valley," a range which roughly covers the limits which in numerous field trips have since been defined more exactly. Dyar records *D. zeacolella* only from points in North Carolina, South Carolina, and Virginia.

To compare larvæ from these sections with the more southern species, the writer obtained specimens from various members of the staff of the Bureau of Entomology from the following places: Columbia and Bennettsville, S. C., and Waynesboro, Ga. (E. R. Barber); Batesburg, S. C. (E. A. McGregor); Thomasville, Ga. (G. D. Smith). All these larvæ were from corn. A casual examination was sufficient to show that they differed from *D. saccharalis crambidoides*. The most apparent difference is that the larvæ (summer form) from the places mentioned above have a clean-cut black-and-white appearance, while larvæ of *D.*

FIG. 1.—*a*, Average angle formed by imaginary lines through bases of setæ of *Diatraea saccharalis crambidoides; b*, average angle formed by imaginary lines through bases of setæ of *D. zeacolella*. Dots indicate bases of setæ.

*saccharalis crambidoides* (summer form), because of the lighter color of the tubercles, are of a more neutral color, which may be described as a kind of dirty white. That the strikingly marked larvæ were *D. zeacolella* was proved by an inflated specimen of the same species in the National Museum, which had been classified by Dr. Dyar as *D. zeacolella*. It was labeled, "On corn, Peacocks Store, N. C."

When the larvæ were shown to Mr. W. Dwight Pierce, of the Bureau of Entomology, he immediately observed a difference in the pattern of the dorsal tubercles (i and ii), the four tubercles of a segment of *D. zeacolella* roughly forming a trapezoid and those of *D. saccharalis crambidoides* forming a more rectangular figure. At the suggestion of Dr. W. D. Hunter, in Charge of Southern Field Crop Insect Investigations, and with the aid of Mr. August Busck, of the Bureau of Entomology, and Rev. J. J. De Gryse, at that time stationed at the Eastern Laboratory of Forest Insect Investigations, the writer proceeded to study the larvæ of the two species for further differences. While the pattern of the dorsal tubercles is valuable, their color fades in the winter, and it is then rather

difficult to determine the exact extent of the tubercles. The writer found that the positions of setæ i and ii correspond with the tubercles of the same numbers and that imaginary lines through the bases of the two setæ on each side form different angles in the two species. By the use of a camera lucida the relative positions of the bases of setæ i and ii on segments 3, 4, and 5 of 19 specimens of *D. saccharalis crambidoides* and of seven specimens of *D. zeacolella* were determined, lines were drawn through the points representing the bases of the setæ, and the resulting angles were measured. It was found that the average angle for *D. saccharalis* was 30.2°, while for *D. zeacolella* it was 53.3°. The size of these angles and the position of the lines through the bases of the setæ are graphically indicated in figure 1.

In different specimens the angles were found to vary from 41° to 69.5° in *D. zeacolella*, and from 18° to 41.5° in *D. saccharalis crambidoides*. Angles of the minimum and maximum numbers of degrees are exceptional.

The differences in the larval characters are noted in the following comparison:

| *Diatraea saccharalis crambidoides* Fabricius. | *Diatraea zeacolella* Dyar. |
|---|---|
| Tubercles light brown or paler (summer form). | Tubercles dark brown, contrasting sharply with ground color of body (summer form). |
| Head brown, but may occasionally be yellow in winter form. | Head yellow. |
| Spiracles dark brown. | Spiracles black. |
| Abdominal tubercles i hardly twice as large as abdominal tubercles ii, more nearly equal. | Abdominal tubercles i about twice as large as abdominal tubercles ii. |
| Abdominal tubercles ii oval, and about twice as far apart as abdominal tubercles i. | Abdominal tubercles ii narrowed and about four times as far apart as abdominal tubercles i. |
| Two imaginary lines connecting bases of setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging 30.2°. | Two imaginary lines connecting bases of setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging 53.3°. |

Descriptions of full-fed larvæ of both the summer and the winter forms of the two species are given below.

**Diatraea saccharalis crambidoides** Fabricius.

SUMMER FORM.—Head rich brown, varying to black at mouth parts and to orange on dorsal aspect; slightly bilobed. Prothoracic plate pale brown, tinged with black ventrally, cephalic third of plate transparent. Body white. Segmentation distinct. Crochets biordinal. Tubercles light brown or paler, iv and v coalesced. Abdominal tubercles ii oval and about twice as far apart as tubercles i. Primary setæ yellow to brown. Imaginary lines connecting setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging 30.2°. No secondary setæ. Spiracles dark brown, elongate oval, distinct. Average length (10 specimens), 25.6 mm.

WINTER FORM.—Head yellow to rich brown, varying to black at mouth parts and to yellow on dorsal aspect; slightly bilobed. Prothoracic plate yellow. Body white. Segmentation distinct. Crochets biordinal. Tubercles white or pale yellow, and not easily distinguished from ground color of body, iv and v coalesced. Abdominal tubercles ii oval and about twice as far apart as tubercles i. Primary setæ yellow to brown. Imaginary lines connecting setæ i and ii, on each side, if prolonged, form angles averaging 30.2°. No secondary setæ. Spiracles dark brown, elongate oval, distinct and sharply contrasting with rest of body. Average length (10 specimens), 22.4 mm.

**Diatraea zeacolella** Dyar.

SUMMER FORM.—Head yellow, varying to black at mouth parts, slightly bilobed. Prothoracic plate yellow. Body white. Segmentation distinct. Crochets biordinal. Tubercles dark brown, contrasting sharply with ground color of body, iv and v coalesced. Abdominal tubercles ii narrowed and about four times as far apart as tubercles i. Primary setæ yellow to brown. Imaginary lines connecting setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging 53.3°. No secondary setæ. Spiracles black, elongate oval, distinct. Average length (3 specimens), 25.2 mm.

WINTER FORM.—Head yellow, varying to black at mouth parts, slightly bilobed. Prothoracic plate yellow. Body white. Segmentation distinct. Crochets biordinal. Tubercles white or pale yellow, and not easily distinguished from ground color of body, iv and v coalesced. Abdominal tubercles ii narrowed and about four times as far apart as tubercles i. Primary setæ yellow to brown. Imaginary lines connecting setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging 53.3°. No secondary setæ. Spiracles black, elongate oval, very distinct and sharply contrasting with rest of body. Average length (4 specimens), 24.5 mm.

The writer has not seen all the instars of *D. zeacolella* Dyar. The instars of *D. saccharalis crambidoides* are similar, except the first, which is described below:

Head black, more horizontal than in following stages. Prothoracic plate dark brown. Body dirty white, widest at head and tapering caudally. Segmentation distinct. Prothoracic legs well developed. Elongate prolegs on protruding coxal lobes. Tubercles prominent. Primary setæ brown. No secondary setæ. Average length about 2 mm.

Not only do the larvæ of the two species vary in appearance, but their food plants and breeding habits differ to some extent. The food plants of *D. saccharalis crambidoides* include sugar cane, corn, and Johnson grass and other large grasses. Practically all of the larval period is spent within the stalks of the plants, except that the first instars feed about on the leaves. *D. zeacolella*, however, seems to have a preference for corn, even where sugar cane is present. Mr. U. C. Loftin found sugar cane at Thomasville, Ga., absolutely uninfested, but Mr. G. D. Smith had no trouble in obtaining larvæ from cornstalks. The writer has examined sugar cane at Waycross, Ga., without finding any larvæ. One larva only was found by Mr. Loftin at Chipley, Fla., in the sugar cane, and this is the only one recorded from sugar cane.

*D. zeacolella* goes far down in the taproots of corn, while *D. saccharalis crambidoides* does not have this habit. This was observed by Mr. E. R.

Barber and has been recorded (under *D. saccharalis*) by Howard[1] and Ainslie.[2]

The fact that *D. saccharalis crambidoides* has been found to be limited to such widely separated areas as southern Florida, southern Louisiana, and the southern tip of Texas will no doubt occasion some surprise. The explanation is that there is strong evidence tending to prove that the species was brought to this country in shipments of sugar cane from the Tropics and that it became established in the three sections in which sugar cane is an important crop. Both forms of larvæ of the two species are well shown in Plate LXXXIX.

[1] Howard, L. O. The larger corn stalk-borer. *In* Insect Life, v. 4, no. 3/4, p. 95–103, fig. 2–4. 1891.
—— The larger corn stalk-borer. U. S. Dept. Agr. Div. Ent. Circ. 16, 3 p., 3 fig. 1896.

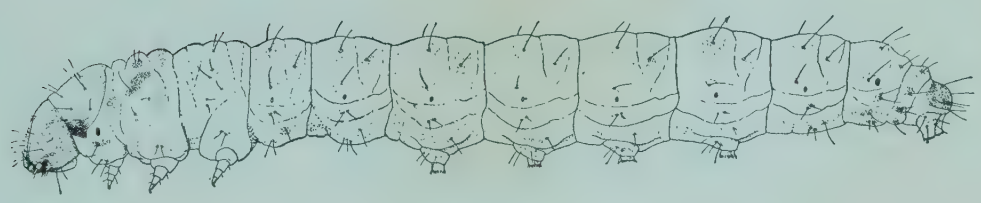
[2] Ainslie, G. G. The larger corn stalk-borer. U. S. Dept. Agr. Farmers' Bul. 634, 8 p., 4 fig. 1914.

## PLATE LXXXIX

Fig. 1.—*Diatraea saccharalis crambidoides*: Larva, summer form, dorsal view.
Fig. 2.—*D. zeacolella:* Larva, summer form, dorsal view.
Fig. 3.—*D. saccharalis crambidoides:* Larva, summer form, side view.
Fig. 4.—*D. zeacolella:* Larva, summer form, side view.
Fig. 5.—*D. saccharalis crambidoides:* Larva, winter form, dorsal view.
Fig. 6.—*D. zeacolella:* Larva, winter form, dorsal view.
Drawn by Mr. Harry Bradford.

1

2



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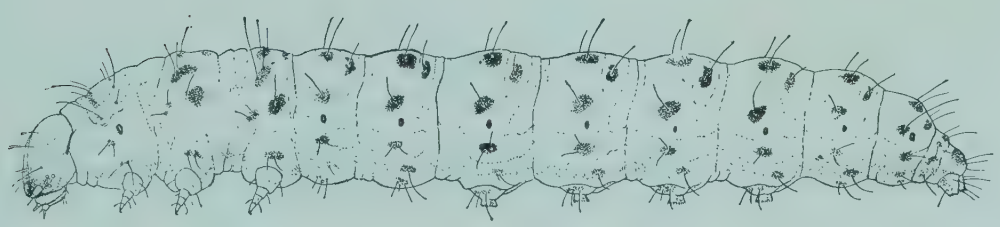

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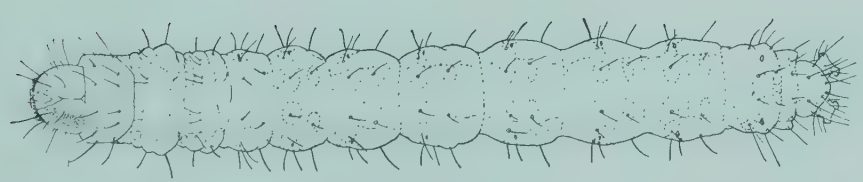

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# THE DISEASE OF POTATOES KNOWN AS "LEAK"[1]

By LON A. HAWKINS,[2]

*Plant Physiologist, Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry*

## INTRODUCTION

The tuber-rot of potatoes (*Solanum tuberosum*) known as the "potato leak" is a disease of considerable importance in the delta region of the San Joaquin River, Cal. The rot is manifest in hot weather and appears soon after harvesting. As the potatoes in this region are sacked in the field and are practically all shipped immediately, the disease is therefore first evident in the car or warehouse. In extreme cases a whole shipment may be so badly damaged as to be worthless. If only a few "leakers" or "melters," as the rotten potatoes are called, are present it is usually necessary to sort the consignment. The cost of this sorting and the attendant shrinkage greatly increase the expense of production.

No exact data could be obtained as to the losses from this disease for any given season, but various estimates placed the damage for 1915 in the whole delta region, in which there were about 40,000 acres of potatoes, between $50,000 and $150,000. The general conditions and the methods of growing potatoes on these peat lands have been described by Orton (11),[3] Irish (8), and Shear (15). Orton and Shear have considered the diseases commonly found on potatoes in that region. In his paper Orton gives the results of a study of the potato leak, which is the only investigation of the disease heretofore reported. He was, however, prevented from completing the work on this disease to his satisfaction, and at his suggestion the writer took up the study.

In the study of the leak of potatoes described in the present paper it was planned to investigate further the causes of the disease, to study the organism or organisms causing it, their mode of entrance into the

[1] The work described in this article was carried out as a part of the potato-disease project of the Office of Cotton and Truck Disease Investigations.

[2] The writer's thanks are due Mr. W. V. Shear, of the Office of Horticultural and Pomological Investigations, for considerable assistance in the work at Stockton, Cal.

[3] Reference is made by number to "Literature cited," p. 639.

tuber, and, if possible, to obtain some data as to methods for its control. Part of the work was carried out at Stockton, Cal., and at various points in the delta potato fields near that city.

## GENERAL APPEARANCE OF THE DISEASE

In taking up the study of the disease in the field, potatoes were examined at the sorting benches in the warehouses at Stockton, and various stages of the disease were observed. It was first apparent as a small brown discoloration around some wound, such as the wound made by the prong of a digging fork or by the breaking off of a "knob," which exposed the tissue of the inner part of the potato. The rot apparently did not affect tubers with unbroken skins. In the later stages of the disease the potatoes were brown over the entire surface, soft, and easily crushed. If sufficient pressure were applied to the tubers, a brownish watery liquid was exuded through breaks in the skin. Sacks containing potatoes in the advanced stages of this disease were frequently wet in patches where the rotten tubers had been crushed against the side. The interior of the rotten potato when broken was usually a dirty white, soon changing to a brown color around the edges. The center generally remained white for some time (Pl. XC).

## ORGANISM CAUSING LEAK

### RHIZOPUS NIGRICANS

Orton proved that the disease was caused by a fungus and concluded that the causal organism was *Rhizopus nigricans* Ehrenb. (10). He based his conclusions on the following premises: He observed a nonseptate mycelium in the rotted tubers and obtained *R. nigricans* in cultures made from these potatoes; he inoculated potatoes with this fungus and produced a rot similar in all appearances to leak.

That *R. nigricans* is able to rot Irish potatoes was also shown in unpublished studies by Mrs. Ethel Field Tillotson. In her experiments she used a strain of *R. nigricans* isolated from sweet potato (*Ipomoea batatas*). Her method of inoculation was to germinate the spores of the fungus in tubes of potato decoction and then pour the liquid, together with the germinated spores, into cavities in the potatoes. The inoculated tubers were placed in damp chambers, and in a few days the disease was evident.

With a strain of *R. nigricans* isolated from sweet potato by Mr. L. L. Harter the present writer was able to inoculate Irish potatoes successfully. The method developed by Mrs. Tillotson was followed in the earlier experiments. It was found unnecessary, however, to germinate the spores before inoculating the potatoes. Accordingly, in the later inoculations the tubers were inoculated directly from a culture of the fungus by inserting some of the spores and mycelium into rather deep wounds made in the tubers with a sterile knife. The inoculated potatoes were then placed in

a moist chamber and in from two to three days about 50 per cent showed evidences of the disease by brown coloration of the skin around the wound. In a week after inoculation the infected potatoes were usually entirely rotted. The skin was brown, and the interior of the potato was soft and watery. They were apparently typical leaky tubers. The fungus was readily reisolated from the rotten potatoes. From the investigations of Orton and the experiments of Mrs. Tillotson and of the present writer it is evident, then, that *R. nigricans* causes a rot of the Irish potato typical in appearance of the disease known as "leak." This work did not prove, however, that all cases of leak were due to *R. nigricans*, as it was very possible that other fungi acting in the same way might produce very similar results.

## PYTHIUM DEBARYANUM

### ISOLATION OF THE FUNGUS

Isolations of the fungus from potatoes were made by transferring portions of the partially rotted tubers obtained in the field to sterile tubes of slanted corn-meal agar and beef agar. In making these transfers the outer surface of the potato which had been washed in a 1 to 1,000 solution of mercuric chlorid was sliced away with a flamed knife and bits of the rotten portion of the potato farthest from the apparent point of infection were removed and placed in the culture tubes. In 24 hours a rather coarse hyalin mycelium was evident on the surface of the agar. After the cultures had grown for three days a microscopic examination of the fungus showed abundant fruiting bodies which much resembled the conidiospores of some species of Pythium. Occasionally structures were found which seemed to be oogonia and antheridia, though these were more frequently seen after a longer period. Transfers were made to the agar slants from 61 typical leaky tubers from a number of different fields. Of these transfers 49 proved to be cultures of this fungus, 5 of which were contaminated with bacteria. Six were cultures of bacteria only, and 6 were sterile. *R. nigricans* was not obtained in any of the cultures.

### MORPHOLOGY OF THE FUNGUS

The fungus obtained from the leaky tubers was studied and found to be apparently a species of Pythium. The mycelium (fig. 1, *c*) of the fungus is rather coarse, irregularly branched, granular, usually nonseptate, though sometimes becoming septate when old. The conidia are borne either terminally or intercalarly. They are usually nearly spherical when mature and are from 12 to 26$\mu$ in diameter, averaging about 22$\mu$. They germinate immediately with one or more germ tubes when they are placed in water at ordinary room temperatures (fig. 1, *d*). The oogonia are spherical and borne like the conidia either terminally or intercalarly.

They are from 15 to 25$\mu$ in diameter, averaging about 22$\mu$. The antheridium (fig. 1, *b*) is borne either on the same filament as the oogonium or on an adjacent filament. If arising from the same filament it may be borne directly below the oogonium or some distance below. More than one antheridium was sometimes found attached to an oogonium. The oospores (fig. 1, *b*) are smooth, spherical, and thick-walled. They are from 14 to 19$\mu$ in diameter, average 16$\mu$, and do not fill the oogonuim. These measurements of the oogonia, oospores, and conidia all agree closely with those of *P. debaryanum*, as given by Butler. A culture of *P. debaryanum* used by Mr. C. P. Hartley in his studies on the damping-off of pine seedlings was obtained from the Office of Forest Pathology. This culture was a subculture of a strain which had been isolated from rotten

FIG. 1.—Microscopical appearance of *Pythium debaryanum* isolated from potatoes affected with potato leak: *a*, cell of a potato tuber showing fungus filaments therein; *b*, oogonia and antheridia; *c*, mycelium; *d*, germinating conidia.

potato by Edson (6) used by him in his studies on seedling diseases of sugar beets and then turned over to Hartley. This fungus agreed very closely with the *Pythium* sp. isolated from the leaky tubers in size of the conidia, oogonia, and oospores, habits of growth, and general appearance. Inoculated into potato tubers it produced a rot similar in all appearances to that produced by the fungus obtained from the leaky potatoes. It would seem, then, from the evidence above cited that the fungus isolated from the leaky potatoes in this study is the same as Hesse's *Pythium debaryanum* (7).

## CULTURAL STUDIES

Cultures of the fungus were made on various kinds of media. The fungus grew well on beef, corn-meal, oatmeal, string-bean, Lima-bean, and potato agars, and Pfeffer's plant agar, potato plugs, and stems of *Melilotus alba*. Conidia and oogonia were formed when the fungus was

grown on string-bean and corn-meal agars, Pfeffer's plant agar, and the stems of *M. alba.* Neither conidia nor oogonia were found when the fungus was grown on the other kinds of media. The fungus produced both sexual and asexual reproductive bodies much more readily in Petri-dish cultures than in tubes. No sporangia or zoospores were seen in any of the cultures made in this study. It is of interest to note that Hesse (7), De Bary (2, 3), Sadebeck (13, 14), and Atkinson (1) are the only writers that to the author's knowledge record having observed the formation of zoospores by this fungus.

Cultures of the fungus were made from single conidia. To make these cultures some of the agar and mycelium from cultures which were producing conidia abundantly was ground up in sterile water. Corn-meal agar plates were poured in the usual way. The conidia germinated usually within an hour. The germinating spores were located by examining the inverted plates with a microscope. They were then marked and removed either to agar slants or to Petri dishes. The growth of these single-spore cultures was similar in all respects to that of the original 49 isolations of this fungus and to that of the strain of *P. debaryanum* obtained from the Office of Forest Pathology. They produced typical conidia, oogonia, and antheridia in abundance, and the mycelium showed the same characteristics as to branching and the granular structure of the protoplasm. Inoculations were made from these cultures into Burbank potatoes with positive results in 90 per cent of the cases. The fungus was reisolated from the rotted potatoes. The results obtained from these single-spore cultures indicate then that only the one fungus, *P. debaryanum,* was present in all the original 49 transfers.

The minimum, optimum, and maximum temperatures for growth and the temperatures at which growth was prevented were roughly determined for this fungus. For these experiments Petri-dish cultures on corn-meal agar were made from subcultures of five different isolations of the fungus and from the strain of *P. debaryanum* obtained from the Office of Forest Pathology. One Petri-dish culture for each constant temperature chamber was inoculated from subcultures from each isolation of the fungus. The growth of the cultures was measured each day for four days, after which the experiment was discontinued, as the culture media in some cases was entirely overgrown with mycelium.

The minimum temperature at which growth was noticeable in four days was between 5° and 8° C. No growth occurred at temperatures below 5°. The temperature at which growth is most rapid lies between 30° and 35°, and the maximum temperature at which growth can occur is between 35° and 40°. The fungus is killed at approximately 40°. Cultures from all five of the isolations from potatoes agreed as to these points, as also did the cultures from the strain of *P. debaryanum* obtained from the Office of Forest Pathology. The fungus was not killed at tem-

peratures below 5°, though growth was inhibited. The cultures from this chamber grew readily when placed in the incubator maintained at 30°. The experiments show that the range of temperature for growth is wide, about 30°, and that the optimum is high. Johnson (9) found the optimum temperature for growth of *P. debaryanum* to be 33°.

### INOCULATION EXPERIMENTS

Inoculations were made into healthy California-grown Burbank potatoes from 30 of the 49 isolations of *P. debaryanum* obtained from diseased potatoes. Tubers were rotted and the fungus reisolated in all cases. Inoculations were also made with the bacterium which was sometimes obtained from the rotten tubers with no apparent effect. It seemed to be present as a saprophyte.

In the earlier inoculation experiments with *P. debaryanum*, the sterile tubers were inoculated with the fungus in wounds made with a flamed knife as in some of the experiments with *Rhizopus nigricans*. The inoculated tubers were then placed in moist chambers. Inasmuch as moist chambers, because of their limited volume of oxygen and their high humidity furnish rather abnormal conditions for the storing of potatoes, another method was developed in which the potatoes were maintained after inoculation under conditions which more nearly approached those found in storage. According to this method, the potatoes were disinfected as before and a small hole made in one side with a sterile knife. A ring, usually the ring of a Van Tieghem cell, was placed over the opening and cemented to the potato with petrolatum. A small quantity of sterile water was poured into the hole in the tuber and the inoculation made by placing some of the mycelium of the fungus in the water. A cover glass was then sealed on top of the cell with petrolatum. Various modifications of this method were tried to determine the size and depth of the wound necessary to insure a high percentage of successful inoculations. It was found that if the skin was removed from a small area of the potato which came within the ring when it was cemented in position and the inoculation made in a drop of sterile water on this wounded area, the results were as good as when deep wounds were made. Further experiments showed that it was sufficient to make a rather deep incision in the tuber with a sterile knife and introduce some mycelium to inoculate the potato successfully. The rots produced by such inoculations, however, became contaminated more frequently with bacteria than when the raw surface of the tuber was inclosed with a ring and cover glass. Numerous controls were prepared by cementing the ring to the unbroken surface of the tuber and placing therein some bits of mycelium in sterile water; also by pouring sterile water into wounds in the potatoes and sealing them as in the inoculation experiments. In none of these controls was there any infection.

In the inoculation experiments 210 sound potatoes of the Burbank variety were used, of which 177, or 84 per cent, were rotted.

Besides the experiments with California-grown potatoes, inoculation experiments were carried out with several eastern-grown varieties. These potatoes were kindly furnished by the Office of Horticulture and Pomological Investigations in most cases. The tubers were inoculated in deep wounds inclosed with a ring and cover glass, according to the method already described. Five different isolations of the fungus were used with each variety of potatoes. After inoculation they were placed in an incubator maintained at 30° C. and left there throughout the experiment. The results of this experiment are shown in Table I.

TABLE I.—*Results of inoculating several varieties of eastern-grown potatoes with Pythium debaryanum, as shown by the number of potatoes of each variety rotted*

| Variety. | Number inoculated. | Number rotted. | Variety. | Number inoculated. | Number rotted. |
|---|---|---|---|---|---|
| Rose 4 (Florida)[1] | 17 | 13 | Early Ohio | 11 | 10 |
| Rose 4 | 9 | 0 | Rural New York | 11 | 7 |
| Early Rose | 12 | 5 | Irish Cobbler | 12 | 5 |
| Triumph | 9 | 5 | Pearl | 12 | 3 |
| Green Mountain | 10 | 7 | | | |

[1] Furnished by Mr. W. B. Clark, of the Office of Cotton and Truck Disease Investigations.

From the results shown in Table I it is evident that some of the varieties of eastern potatoes are about as susceptible to this disease as California-grown Burbanks used in the experiments already described. Early Ohio was apparently most susceptible, in that 10 potatoes rotted out of 11 inoculated. The other varieties seemed somewhat more resistant to this disease.

Inoculations were also made, using potatoes of undetermined varieties purchased in the Washington markets. A fair percentage of these inoculations were successful in all cases. It would seem, then, that susceptibility to this disease is not necessarily confined to potatoes grown on the peat lands of California.

Another series of inoculation experiments was carried out to ascertain what temperatures were most favorable for the growth of the fungus in the potato and at what temperatures no infection would result from an inoculation. In these experiments inoculated potatoes were kept at seven different temperatures, varying in 5-degree intervals from 5° to 35° C. Seventy potatoes of the Burbank variety were used. Forty of these potatoes, those intended for the lower temperatures, were kept in the ice box at about 10° C. for 24 hours before inoculation, so that their temperature at the time of inoculation would be more nearly that at which they were to be maintained during the experiments. For the inoculations

subcultures from five separate isolations of the fungus were used, and 14 potatoes were inoculated from subcultures from each isolation, 2 for each of the constant-temperature chambers. They were maintained at constant temperatures for one week and were then removed and examined. The results of these experiments are shown in Table II.

TABLE II.—*Results of experiments in which inoculated potatoes were maintained at constant temperatures, 10 Burbank potatoes in each chamber, maintained at constant temperature for one week. All sound potatoes were then placed in the 30° chamber for three days*

| Temperature. | Number of tubers showing infection in one week. | Number of tubers which did not show evidences of infection in chambers originally used but which were rotted three days after removal to 30° chamber. | Total number of rotted potatoes. |
|---|---|---|---|
| °C. | | | |
| 5 | 0 | 7 | 7 |
| 10 | 3 | 2 | 5 |
| 15 | 7 | 2 | 8 |
| 20 | 8 | 0 | 8 |
| 25 | 9 | 0 | 9 |
| 30 | 10 | 0 | 10 |
| 35 | 10 | 0 | 10 |

The results given in Table II show that a higher percentage of inoculated potatoes are rotted at temperatures near the optimum for growth of the fungus in artificial culture media than at the lower temperatures. It is evident, however, that temperatures near this optimum are not necessary for infection. As was to be expected, no rot was produced while the inoculated potatoes were maintained at 5° C., but when these potatoes were moved from this chamber to the incubator maintained at 30°, 70 per cent of them were rotted in three days. The growth of the fungus is apparently inhibited at the low temperature, but begins as soon as the temperature is raised. The lowest total amount of rot was in the potatoes maintained at 10° for the week. In this case 50 per cent of the inoculated potatoes rotted. The growth of the fungus in the potato is slower at the lower temperatures, 10° and 15°, than at the higher temperatures, as was found to be the case with this fungus on artificial-culture media.

It is evident from these experiments in which *P. debaryanum* was isolated from 49 diseased tubers, inoculations made from 30 of these isolations into healthy tubers, the disease produced, and the fungus subsequently reisolated that this fungus is frequently present in potatoes affected with leak and that when inoculated into the tubers, it causes this rot.

### GROWTH OF PYTHIUM DEBARYANUM IN THE TUBER

The rate of growth of the fungus in the potato was approximately determined. A Green Mountain potato which had been inoculated in the usual way and allowed to remain at 30° C. for 67 hours was sliced open. The fungus was found to have penetrated to a depth of 4 cm. from the point of inoculation during this time. The average diameter of the cell of the potato, obtained by measuring a large number of cells, was found to be 138.7$\mu$. By calculation the fungus must have passed through approximately 288 cells in 67 hours, or at the rate of 1 cell every 14 minutes. This calculation does not take into account the period of readjustment of the fungus before it begins to grow into the tissue of the potato, which is probably appreciable.

Portions of a potato tuber which had been rotted with *P. debaryanum* were killed, embedded in paraffin, sectioned, and stained.[1] Examination of these sections showed that the mycelium was distributed quite generally throughout the tissue of the host. It usually passes directly through the cell wall (fig. 1, *a*) and through the lumen of the cell, though it was found occasionally between the cells. It branches frequently. Where the hypha of the fungus passes through the cell wall, it is markedly constricted (fig. 1, *a*). Ward (16), in his work on this fungus, also observed that the opening made in the cell wall was smaller than the mean diameter of the fungus hyphæ. Rosenbaum (12) shows the same relation between cell wall of host plant and fungus hypha in his work with *Phytophthora cactorum* on ginseng.

### INFECTION OF POTATOES FROM SOIL

It was mentioned earlier in this paper that the disease was observed only in potatoes which had been wounded. In inoculation experiments it was never possible to cause the disease without first breaking the skin of the potato. The wounds observed in the rotting potatoes in the field studies had been made when the potatoes were harvested, which leads to the conclusion that the organisms causing the leak are probably present in the soil and are introduced into the freshly wounded potato in digging. To obtain evidence on this point, Petri-dish cultures on corn-meal agar were made from samples of the peat soils from various parts of the delta potato region. *P. debaryanum* was found in every case. Inoculations were made by inserting some of the soil into holes in the tubers and in about 50 per cent of the cases the tubers were rotted. *P. debaryanum* was isolated from the rotted tubers.

Field tests were made on the effect of wounding the potatoes in digging. In these experiments seven sacks, or about 12 bushels, of potatoes were harvested. The work was done rather carelessly so that many tubers were injured with the digging forks. The sound potatoes were

[1] The writer is indebted to Mr. Charles S. Ridgway, of the Office of Tobacco Investigations, for the making and staining of these slides. They were stained in methylene blue-eosin combination which leaves the fungus hyphæ bright blue and the cell walls of the host plant red.

sorted out and sacked separately, and all the potatoes were stored in sacks in a warehouse under about the usual commercial conditions. The potatoes were sorted four days later and 65 diseased tubers were found, all of which had been wounded. They were sorted a second time eight days after digging and 52 more rotten tubers were found. None of the unwounded potatoes showed evidences of the disease at any time, and no more of the wounded tubers were rotten when they were sorted for the last time 15 days after harvesting. Transfers were made from some of these rotten tubers to corn-meal agar slants and *P. debaryanum* was obtained in all these cultures. It is evident that this fungus is generally present in these peat soils, that inoculations may be made by inserting some of the soil in wounds in the tubers, and that potatoes wounded in digging frequently become infected. Unwounded tubers are apparently not affected with this disease. It would seem probable from these experiments that more care in harvesting and sorting out of potatoes injured in digging would decrease the losses from this disease.

## OTHER ROTS SOMETIMES MISTAKEN FOR LEAK

It is quite possible that tuber-rots produced by other fungi may be mistaken for potato leak. Two species of Fusarium, *F. radicicola* Wollenw. and *F. oxysporum* Schlecht., which produce tuber-rots of the potato are quite common in the San Joaquin potato region. Carpenter (5) has shown that either one or the other of these fungi is usually present in the jelly-end rot of potato tubers. He has also obtained *F. radicicola* from specimens of rotten potatoes from San Joaquin County, California, which were supposed to be affected with leak. The present writer has found rotten tubers in consignments of potatoes from California which had stood in the laboratory for a few weeks. These potatoes were apparently sound upon arrival, with the exception of a few which had small rotten spots in the stem end. At the end of a few weeks some of the tubers were entirely rotten and very much resembled those in the advanced stages of leak. *F. radicicola* was obtained from several such specimens. Neither *Pythium debaryanum* nor *Rhizopus nigricans* was ever obtained. Potato dealers at Stockton and potato growers say that the leak may develop after the potatoes have been in storage for a time and sometimes after they have been sorted. Under such conditions the rot is apparently not due to *P. debaryanum* nor *R. nigricans*, but to some other organism, probably a species of Fusarium in many instances, as in these experiments rots caused by *P. debaryanum* or *R. nigricans* were usually evident in three or four days. If an inoculated potato was sound at the end of a week it was not infected and the potato would remain sound indefinitely. The experiments in which potatoes were kept at low temperatures are, of course, excepted. It seems quite probable then that potatoes affected with rots caused by *Fusarium* spp. are sometimes confused with those affected with leak.

## DISCUSSION OF RESULTS

It is evident from the experiments described in this paper and from the work that has been done heretofore that *R. nigricans* rots potato tubers. That it is the cause of a rot of potatoes under field and warehouse conditions has been shown by Orton (10). From the experiments carried out in this study, however, it seems that potato leak is most commonly caused by *P. debaryanum*. At least this seems to have been the case during the season of 1915.

When inoculated into potatoes, both fungi rot the tubers either very rapidly or not at all. It seems that if the disease is not well advanced in a week at 30° C. the potato is not infected. The rots produced by these fungi have practically the same general appearance.

The parasitism of *P. debaryanum* on seedlings of various plants is too well known to require discussion here. That it should be the cause of a potato disease of considerable importance is not surprising when the work of earlier writers is taken into account. Sadebeck (14), in 1875, reported the discovery of a species of Pythium parasitic upon potato plants near Coblenz. He considered the fungus to be *P. equiseti* Sadebeck. He mentions finding it on various parts of the plants. That *P. equiseti* was identical with *P. debaryanum* was later pointed out by De Bary (2). De Bary in some of his experiments grew *P. debaryanum* on living potato tubers. Ward (16) also cultivated it on this host and considered potatoes ". . . a very good medium for the cultivation of the fungus." Edson (6) recently obtained this fungus from rotten potato tubers. No one seems to have succeeded in inoculating any part of the potato plant except the tuber with this organism.

That this fungus should cause so much damage to potatoes in the San Joaquin delta region is probably largely due to the conditions and methods of handling the potatoes in that section. As has been said, the potatoes are dug with forks, and many are wounded in the process. Potatoes with branches, or "knobs," are quite common, and these branches are usually broken off in harvesting, if the potato is of marketable size, and the main tuber retained. Perhaps the broken surface of the tuber is rubbed in the soil, "to dry it." That these are excellent methods for inoculating potatoes with *P. debaryanum* has been shown. The potatoes are sacked as soon as dug. They may then stand in the sun for some hours before they are hauled to the car or boat landing for shipment. In the car or on the boat the sacks are usually piled up. The humidity among these tubers is, of course, high because of the high rate of transpiration. This, together with the relatively high temperature, offers good conditions for the development of any parasitic fungus, such as *P. debaryanum*, with which the tubers may have been inoculated. It is quite possible that the leak of potatoes would have been reported from other localities where either *R. nigricans* or *P. debaryanum* are common in the

soils if the methods of harvesting and handling and the temperature conditions were as favorable for the development of these parasites as they are in the delta region of the San Joaquin River.

It is considered by the potato growers of this region that the disease is much more common in hot weather. In these experiments it was shown that the optimum temperature for growth of the fungus is high (between 30° and 35° C.) and that the fungus infects the potatoes more readily at temperatures near this optimum. At the lower temperatures the percentage of infection is not so high, and the growth of the fungus is retarded or, as in the case of the experiments at 5°, inhibited while the potatoes remained at that temperature. It would seem then that lowering the temperatures of the cars and storage warehouse might retard the development of the disease, but that the infected potatoes would rot as soon as the temperature was raised. From the data now at hand, icing the cars and cold storage of the potatoes would seem to be of doubtful value as control measures. The control of the disease seems more likely to lie along the lines of better methods of harvesting and handling, as Orton suggested (10), and a careful sorting out of all wounded tubers.

## CONCLUSIONS

In the work described in this paper the conclusion of Orton that *Rhizopus nigricans* Ehrenb. can cause a rot of potatoes has been corroborated. This fungus was not, however, isolated in the field experiments from tubers affected with leak. A fungus was obtained 49 times in 61 attempts. The cultures were made from a different tuber each time. A study was made of the fungus and it was found to be *Pythium debaryanum* Hesse. In inoculation experiments this fungus produced a rot typical in all appearances to the potato leak, and was readily reisolated from the diseased tuber. It seems probable that the disease is produced by both *R. nigricans* and *P. debaryanum*. The latter is apparently more frequently the causal organism.

*P. debaryanum* was found in soil samples taken from various parts of the delta potato region. The disease was produced by inserting some of this soil in wounds in the potato tubers and *P. debaryanum* was isolated from these rotted potatoes. Infection apparently takes place in the field by some of this infected soil getting into wounds made in digging. No cases of infection were observed either under field conditions or in the laboratory where the skin of the tuber was unbroken. From the results of these experiments it seems that the disease might be controlled by more care in harvesting and handling the potatoes and a careful sorting out of all wounded tubers.

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## PLATE XC

Potatoes affected with potato leak:

Fig. 1, 2.—Natural infection from fork wound; photographed by Dr. W. A. Orton.
Fig. 3.—Rot produced by inoculation with *Pythium debaryanum.*
Fig. 4.—Rot produced by inoculation with *Rhizopus nigricans.* Inoculation made by Mrs. Tillotson.

# DIGESTIBILITY OF HARD PALATES OF CATTLE

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The so-called "hard palates," which are taken from the roof of the mouth of beef animals, have not in the past been utilized to any extent as food. They contain very little muscular tissue, such as is characteristic of meats in general, and possess a ribbed outer surface that is black or white in color, very rough, and of an unattractive appearance.

The microscopic examination of the structural constituents of hard palates of cattle reveals a stratified layer of epithelium which is in a state of cornification. The extent of this layer is possibly one-sixteenth of the entire thickness. The connective tissue portion of the mucous membrane consists of a dense feltwork of white fibrous tissue arranged in dense interlacing bundles; the individual fibers of the bundles, comprising about 60 per cent, are matted together as closely as in tendon tissue or sinews and are interwoven with about 20 per cent of elastic (erectile) fibers, 10 per cent of involuntary muscle, and about 10 per cent of looser fibrous tissue attaching the mucous membrane to the periosteum. This looser tissue contains a small amount of fat and very few glands.

A chemical examination of hard palates showed that when freshly procured they have the following composition: Water, 71.0 per cent; protein (N×6.25), 22.2 per cent (or protein by difference, 16.6 per cent); fat, 11.8 per cent; and ash, 0.6 per cent. The high protein content suggested that this material might be of value for food. Since little, if any, experimental evidence is available regarding the thoroughness of digestion of such tissue when eaten in quantity, a number of experiments were undertaken at the suggestion of the Bureau of Animal Industry to determine the digestibility of hard palates by human subjects. This means for practical purposes the digestibility of the nitrogenous material present, since the proportion of fat supplied by the cooked hard palates is small.

## COOKING HARD PALATES

The material for study was obtained from a local abattoir and supplied to the Office of Home Economics by the Bureau of Animal Industry. Before the digestion experiments could be undertaken, it was necessary to find some way of cooking and serving the hard palates which would make it possible to eat them in quantity. At first the attempt was made to put the raw material through an ordinary household meat cutter with the idea that it might then be fried in small cakes, like Hamburg steak, but the material was so firm and tough that it

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could not be minced in this way. Accordingly it was decided to cook the palates before trying to mince them, and tests showed that after boiling for two or three hours they could be easily minced with a meat cutter and that so prepared the texture as well as the flavor was not disagreeable, particularly if the palates were combined with other food materials.

The average composition of the cooked palates was found to be as follows: Water, 71.1 per cent; protein (N×6.25), 21.8 per cent (or protein by difference, 22.3 per cent); fat, 6.3 per cent; and ash, 0.3 per cent. The material used for analysis weighed before cooking 15½ ounces and after cooking 14 ounces, the total loss therefore being only 1½ ounces. As will be noted by referring to the percentage composition of the raw material, the boiled palates had, in round numbers, only one-half the fat, one-half the ash, and nine-tenths the protein content of the fresh material. As found by analysis, 50.0 per cent of the ash, 46.6 per cent of the fat, and 11.3 per cent of the protein originally present were removed by cooking. In general, the observed effects are in accord with Grindley's observations[1] that, except for a lowered fat and ash content and the removal of some soluble nitrogenous material, cooked meat has very much the same proximate composition as it has raw.

The water in which the palates were boiled did not look at all like that in which beef is cooked, but was white in color and not unlike milk in appearance. The character of the nitrogenous constituents present was not studied in detail, but preliminary tests indicated that gelatin predominated, with traces of coagulable albumin, globulin, and primary proteoses.

Some attention was given to the hard palate fat which floated to the top of the liquor in which the palates were boiled. This hardened on cooling and was purified by remelting several times to remove the sediment. The product had a deep-yellow color, a mild flavor, and an appearance suggesting butter, though rather more granular. It was found to have a melting point of 34° C., an iodin number of 52.53, and a refractive index of 1.4586.[2] The amount obtained was not sufficient for further study.

The cooked palates had a mild and not unpleasant flavor and in appearance resembled cooked gristle or connective tissue rather than lean meat, this resemblance being noticeable even when the material was finely ground. It was apparent that the cooked palates would be much more acceptable as the principal constituent of the experimental ration if prepared in some savory form, and meat cakes and meat loaf naturally suggested themselves as possibilities. The meat cakes

[1] Grindley, H. S., and Mojonnier, Timothy. Experiments on losses in cooking meat, 1900–1903. U. S. Dept. Agr. Office Exp. Stas. Bul. 141, p. 94. 1904.

[2] Information regarding the structure and composition of the hard palates and the chemical nature of the material extracted during cooking was supplied by the Bureau of Animal Industry.

did not prove satisfactory, having, when thoroughly cooked and well browned, a flavor suggesting that of scorched or burned gristle or bone. On the other hand, meat loaf made according to a common household recipe and containing in addition to the hard palates some flour, butter, and onions, and sweet herb, salt, and pepper as seasoning was found to be satisfactory for the purpose. The flour served to bind the material together so that the loaf would retain its shape and could be sliced without crumbling, while the butter improved both the texture and the flavor.

## EXPERIMENTAL RATION

Experience has shown that the normal individual eats more heartily of a food material if it forms a part of a mixed ration than if it is the only food served for several successive meals. Accordingly, with the meat loaf made from hard palates, a uniform basal ration simple in character (crackers and butter, boiled potatoes, and tea or coffee with sugar but no milk or cream) was served. A basal ration which obviously contained only a minimum amount of protein was selected, in order that the hard palates might supply the greater part of the protein of the experimental diet. In making a quantity of the meat loaf sufficient for a three-day digestion experiment for four subjects the following quantities were used: Boiled hard palates finely minced, 13½ pounds; flour, 1 pound; butter, ½ pound; onions, 3 of medium size; and seasoning (sage, salt, and pepper to taste).

## METHODS OF DIGESTION EXPERIMENTS

Four subjects who had gained experience in this type of work in the study of the digestibility of other foods assisted in this investigation. They were young men of medium weight and of good health, moderately active, and sufficiently informed through previous experience to appreciate the importance of observing accuracy in following all directions given them.

As is evident from a consideration of their composition and the amounts eaten, hard palates supplied only a small part of the total fat of the experimental ration and a very little ash. Furthermore, since little, if any, carbohydrate was present in the hard palates, it follows that interest centers on the digestibility of protein, since this is the only food constituent which they provide in quantity.

Experience has shown that it is desirable to supply a food constituent in generous proportions in order that the calculated coefficients of digestibility may not be masked by unavoidable errors incidental to the methods followed. To make sure that the amount of protein eaten was generous, a fairly large allowance of the meat loaf made from hard palates was served at each meal and the subjects were urged to eat all of it. At the same time, as already noted above, only a limited amount of protein was obtainable from other sources.

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As regards the experimental details, the methods followed in studying the hard palates were similar to those previously reported with other foods.[1] As no attempt was made to maintain body weight or to approximate a nitrogen equilibrium, the quantity of the entire ration to be eaten was not stipulated. The feces occurring from each experimental period, as indicated by charcoal markers, were collected and dried to remove the water. Samples of foods eaten were retained for analysis and all analyses of foods and feces were made by the methods described by the Association of Official Agricultural Chemists.[2]

In order to determine the digestibility of a single food contained in a mixed diet, it is necessary either to determine the digestibility of the basal ration and to apply the proper correction to the values obtained for the digestibility of the total diet, or to estimate the undigested residue occurring from the various constituents of the diet by means of coefficients previously determined, and to make proper allowance for this undigested material. The latter method has been followed in this instance and the method of estimating the digestibility of the protein of the meat loaf alone is indicated by the following equations:

[Weight of protein in potato, crackers, and butter]×[Percentage of undigested protein occurring in each]=[Weight of undigested protein present in feces derived from basal ration].

[Total undigested protein in feces]−[Undigested protein in feces from basal ration]=[Undigested protein occurring from meat loaf].

[(Total protein of meat loaf)−(Undigested protein from meat loaf)]÷[Total protein of meat loaf]=[Estimated percentage digestibility of meat loaf alone].

On the basis of determinations by previous investigators the coefficients assumed in these equations for the digestibility of the protein of the potatoes, crackers, and butter are 83 per cent,[3] 93.8 per cent,[4] and 97 per cent,[3] respectively.

In Table I are recorded the essential experimental data of the digestion experiments with hard palates, including the total weight of food eaten, the nutrients furnished, the weight of feces, the undigested nutrients therein, the percentage of the different nutrients digested, and the estimated digestibility of the protein of the meat loaf.

[1] Langworthy, C. F., and Holmes, A. D. Digestibility of some animal fats. U. S. Dept. Agr. Bul. 310, 22 p. 1915.

[2] Wiley, H. W. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted in 1912.

[3] Atwater, W. O., and Bryant, A. P. The availability and fuel value of food materials. *In* Conn. Storrs Agr. Exp. Sta. 12th Ann. Rpt., 1899, p. 104. 1900.

[4] Woods, C. D., and Merrill, L. H. Studies on the digestibility and nutritive value of bread at the Maine agricultural experiment station, 1899–1903. U. S. Dept. Agr. Office Exp. Stas. Bul. 143, p. 33. 1904.

TABLE I.—*Results of digestion experiments with hard palates of cattle in a simple mixed diet*

| Item. | Weight of food. | Water. | Protein. | Fat. | Carbohydrates. | Ash. |
|---|---|---|---|---|---|---|
| Experiment 334 (subject H. F. B.): | | | | | | |
| Hard palates (in form of meat loaf).....gm.. | 1,309 | 749.3 | 317.4 | 141.2 | 71.4 | 29.7 |
| Potato.....gm.. | 1,242 | 937.7 | 31.1 | 1.2 | 259.6 | 12.4 |
| Crackers.....gm.. | 670 | 40.0 | 56.5 | 96.5 | 472.3 | 4.7 |
| Butter.....gm.. | 261 | 28.7 | 2.6 | 221.9 | ..... | 7.8 |
| Sugar.....gm.. | 213 | ..... | ..... | ..... | 213.0 | ..... |
| Total food consumed, gm..... | 3,695 | 1,755.7 | 407.6 | 460.8 | 1,016.3 | 54.6 |
| Feces.....gm.. | 136 | ..... | 72.3 | 18.8 | 32.1 | 12.8 |
| Amount utilized....gm.. | ..... | ..... | 335.3 | 442.0 | 984.2 | 41.8 |
| Digestibility of entire ration.....per cent.. | ..... | ..... | 82.3 | 95.9 | 96.8 | 76.6 |
| Estimated digestibility of meat loaf.....per cent.. | ..... | ..... | 80.0 | ..... | ..... | ..... |
| Experiment 335 (subject D. G. G.): | | | | | | |
| Hard palates (in form of meat loaf).....gm.. | 1,327 | 759.6 | 321.8 | 143.2 | 72.3 | 30.1 |
| Potato.....gm.. | 1,232 | 930.2 | 30.8 | 1.2 | 257.5 | 12.3 |
| Crackers.....gm.. | 661 | 39.5 | 55.8 | 95.2 | 465.9 | 4.6 |
| Butter.....gm.. | 313 | 34.4 | 3.1 | 266.1 | ..... | 9.4 |
| Sugar.....gm.. | 111 | ..... | ..... | ..... | 111.0 | ..... |
| Total food consumed, gm..... | 3,644 | 1,763.7 | 411.5 | 505.7 | 906.7 | 56.4 |
| Feces.....gm.. | 105 | ..... | 54.5 | 21.0 | 20.0 | 9.5 |
| Amount utilized....gm.. | ..... | ..... | 357.0 | 484.7 | 886.7 | 46.9 |
| Digestibility of entire ration.....per cent.. | ..... | ..... | 86.8 | 95.8 | 97.8 | 83.2 |
| Estimated digestibility of meat loaf....per cent.. | ..... | ..... | 85.8 | ..... | ..... | ..... |
| Experiment 336 (subject R. L. S.): | | | | | | |
| Hard palates (in form of meat loaf).....gm.. | 1,329 | 760.7 | 322.3 | 143.4 | 72.4 | 30.2 |
| Potato.....gm.. | 1,275 | 962.6 | 31.9 | 1.3 | 266.5 | 12.7 |
| Crackers.....gm.. | 291 | 17.4 | 24.6 | 41.9 | 205.1 | 2.0 |
| Butter.....gm.. | 208 | 22.9 | 2.1 | 176.8 | ..... | 6.2 |
| Sugar.....gm.. | 69 | ..... | ..... | ..... | 69.0 | ..... |
| Total food consumed, gm..... | 3,172 | 1,763.6 | 380.9 | 363.4 | 613.0 | 51.1 |
| Feces.....gm.. | 70 | ..... | 32.8 | 15.9 | 13.4 | 7.9 |
| Amount utilized.....gm.. | ..... | ..... | 348.1 | 347.5 | 599.6 | 43.2 |
| Digestibility of entire ration.....per cent.. | ..... | ..... | 91.4 | 95.6 | 97.8 | 84.5 |
| Estimated digestibility of meat loaf.....per cent.. | ..... | ..... | 92.0 | ..... | ..... | ..... |

TABLE I.—*Results of digestion experiments with hard palates of cattle in a simple mixed diet*—Continued

| Item. | Weight of food. | Water. | Protein. | Fat. | Carbohydrates. | Ash. |
|---|---|---|---|---|---|---|
| Experiment 337 (subject O. E. S.): | | | | | | |
| Hard palates (in form of meat loaf).....gm.. | 1,052 | 602.2 | 255.1 | 113.5 | 57.3 | 23.9 |
| Potato.....gm.. | 1,126 | 850.1 | 28.2 | 1.1 | 235.3 | 11.3 |
| Crackers.....gm.. | 525 | 31.3 | 44.3 | 75.6 | 370.1 | 3.7 |
| Butter.....gm.. | 258 | 28.4 | 2.6 | 219.3 | ..... | 7.7 |
| Sugar.....gm.. | 467 | ..... | ..... | ..... | 467.0 | ..... |
| Total food consumed, gm..... | 3,428 | 1,512.0 | 330.2 | 409.5 | 1,129.7 | 46.6 |
| Feces.....gm.. | 98 | ..... | 41.4 | 31.7 | 14.7 | 10.2 |
| Amount utilized.....gm.. | ..... | ..... | 288.8 | 377.8 | 1,115.0 | 36.4 |
| Digestibility of entire ration.....per cent.. | ..... | ..... | 87.5 | 92.3 | 98.7 | 78.1 |
| Estimated digestibility of meat loaf.....per cent.. | ..... | ..... | 86.8 | ..... | ..... | ..... |
| Experiment 342 (subject H. F. B.): | | | | | | |
| Hard palates (in form of meat loaf).....gm.. | 1,312 | 755.2 | 336.2 | 118.5 | 86.8 | 15.3 |
| Potato.....gm.. | 1,216 | 918.1 | 30.4 | 1.2 | 254.1 | 12.2 |
| Crackers.....gm.. | 309 | 21.3 | 25.0 | 41.4 | 218.8 | 2.5 |
| Butter.....gm.. | 107 | 11.8 | 1.1 | 90.9 | ..... | 3.2 |
| Sugar.....gm.. | 317 | ..... | ..... | ..... | 317.0 | ..... |
| Total food consumed, gm..... | 3,261 | 1,706.4 | 392.7 | 252.0 | 876.7 | 33.2 |
| Feces.....gm.. | 97 | ..... | 54.4 | 14.1 | 19.9 | 8.6 |
| Amount utilized.....gm.. | ..... | ..... | 338.3 | 237.9 | 856.8 | 24.6 |
| Digestibility of entire ration.....per cent.. | ..... | ..... | 86.1 | 94.4 | 97.7 | 74.1 |
| Estimated digestibility of meat loaf.....per cent.. | ..... | ..... | 85.8 | ..... | ..... | ..... |
| Experiment 343 (subject D. G. G.): | | | | | | |
| Hard palates (in form of meat loaf).....gm.. | 1,473 | 847.9 | 377.5 | 133.0 | 97.4 | 17.2 |
| Potato.....gm.. | 1,176 | 887.9 | 29.4 | 1.2 | 245.8 | 11.7 |
| Crackers.....gm.. | 376 | 25.9 | 30.5 | 50.4 | 266.2 | 3.0 |
| Butter.....gm.. | 288 | 31.7 | 2.9 | 244.8 | ..... | 8.6 |
| Sugar.....gm.. | 96 | ..... | ..... | ..... | 96.0 | ..... |
| Total food consumed, gm..... | 3,409 | 1,793.4 | 440.3 | 429.4 | 705.4 | 40.5 |
| Feces.....gm.. | 93 | ..... | 50.3 | 15.5 | 19.6 | 7.6 |
| Amount utilized.....gm.. | ..... | ..... | 390.0 | 413.9 | 685.8 | 32.9 |
| Digestibility of entire ration.....per cent.. | ..... | ..... | 88.6 | 96.4 | 97.2 | 81.2 |
| Estimated digestibility of meat loaf.....per cent.. | ..... | ..... | 88.5 | ..... | ..... | ..... |

TABLE I.—*Results of digestion experiments with hard palates of cattle in a simple mixed diet*—Continued

| Item. | Weight of food. | Water. | Protein. | Fat. | Carbohydrates. | Ash. |
|---|---|---|---|---|---|---|
| Experiment 345 (subject O. E. S.): | | | | | | |
| Hard palates (in form of meat loaf).....gm.. | 1,296 | 746.0 | 332.1 | 117.0 | 85.7 | 15.2 |
| Potato.....gm.. | 1,009 | 761.8 | 25.2 | 1.0 | 210.9 | 10.1 |
| Crackers.....gm.. | 345 | 23.8 | 27.9 | 46.2 | 244.3 | 2.8 |
| Butter.....gm.. | 104 | 11.5 | 1.0 | 88.4 | ..... | 3.1 |
| Sugar.....gm.. | 340 | ..... | ..... | ..... | 340.0 | ..... |
| Total food consumed, gm..... | 3,094 | 1,543.1 | 386.2 | 252.6 | 880.9 | 31.2 |
| Feces.....gm.. | 98 | ..... | 45.3 | 20.4 | 22.1 | 10.2 |
| Amount utilized.....gm.. | ..... | ..... | 340.9 | 232.2 | 858.8 | 21.0 |
| Digestibility of entire ration.....per cent.. | ..... | ..... | 88.2 | 91.9 | 97.5 | 67.3 |
| Estimated digestibility of meat loaf.....per cent.. | ..... | ..... | 88.2 | ..... | ..... | ..... |
| Average food consumed per subject per day.gm.. | 1,129 | 563.7 | 130.9 | 127.3 | 291.8 | 14.9 |

SUMMARY

| Experiment No. | Subject. | Digestibility of entire ration (per cent). | | | | Estimated digestibility of protein of meat loaf alone (per cent). |
|---|---|---|---|---|---|---|
| | | Protein. | Fat. | Carbohydrates. | Ash. | |
| 334 | H. F. B..... | 82.3 | 95.9 | 96.8 | 76.6 | 80.0 |
| 335 | D. G. G..... | 86.8 | 95.8 | 97.8 | 83.2 | 86.8 |
| 336 | R. L. S..... | 91.4 | 95.6 | 97.8 | 84.5 | 91.9 |
| 337 | O. E. S..... | 87.5 | 92.3 | 98.7 | 78.1 | 86.7 |
| 342 | H. F. B..... | 86.1 | 94.4 | 97.7 | 74.1 | 85.8 |
| 343 | D. G. G..... | 88.6 | 96.4 | 97.2 | 81.2 | 88.5 |
| 345 | O. E. S..... | 88.2 | 91.9 | 97.5 | 67.3 | 88.2 |
| | Average..... | 87.3 | 94.6 | 97.6 | 77.9 | 86.8 |

The average amount of food eaten per subject per day was 1,129 gm. which furnished 564 gm. of water, 131 gm. of protein, 127 gm. of fat, 292 gm. of carbohydrates, and 15 gm. of ash. The uniformity of values obtained in the different experiments for the digestibility of the carbohydrates and the close agreement of the average value, 97.6 per cent, with the value, 97 per cent, given for the digestibility of carbohydrates in the ordinary mixed diet[1] would indicate that care had been observed in the collection of the feces. The digestibility of fat is of interest, in that practically all of the fat of the diet was obtained from the butter,

[1] Atwater, W. O. On the digestibility and availability of food materials. *In* Conn. Storrs Agr. Exp. Sta. 14th Ann. Rpt., 1901, p. 245. 1902.

part of which was present as a constituent of the meat loaf and a part as a constituent of the basal ration, supplying in all approximately 125 gm. of fat per subject per day. This was 94.6 per cent assimilated, which for all practical purposes is identical with the digestibility of butter found in a previous investigation,[1] 93.9 per cent.

Inasmuch as the subjects were allowed to eat of the basal ration according to individual preferences, the energy value of the diet was not uniform. It was found, however, that the subjects eating as much as they wished received, on an average, 3,265 Calories daily, calculated from the average daily consumption of protein, fat, and carbohydrates, and the factors [2] commonly used in the determination of fuel values. In view of the fact that over 130 gm. of protein, largely supplied by the meat loaf, and over 3,200 Calories of energy were consumed daily, it is apparent that the ration was eaten with relish.

The digestibility of the total protein of the diet was found to be 87.3 per cent. The meat loaf supplied 82 per cent of the total protein consumed, a much larger proportion than is ordinarily furnished by the meat portion of a meal; consequently, greater accuracy is possible in estimating the digestibility of the protein contained in the meat loaf.

The digestibility of the protein of the meat loaf alone, 86.8 per cent, differs little from the value of the digestibility of the entire ration. This is due partly to the rather complete assimilation of the protein of the basal ration and partly to the relatively small amount of protein derived from this source. The value, 86.8 per cent, represents the digestible protein of the meat loaf, but it should closely approximate that for the protein of the hard palates, since in the preparation of the loaf the proportions used were 13.5 parts of hard palates to 1 part of flour. An allowance may be made for the flour by assuming the protein from this source to be 93.8 per cent [3] digestible. From the results of this investigation, accordingly, it would seem that the protein of hard palates which have been thoroughly cooked is somewhat less thoroughly assimilated than that of the common cuts of meat.[4]

[1] Langworthy, C. F., and Holmes, A. D. Digestibility of some animal fats. U. S. Dept. Agr. Bul. 310, 22 p. 1915.

[2] Atwater, W. O., and Bryant, A. P. The availability and fuel value of food materials. *In* Conn. Storrs Agr. Exp. Sta. 12th Ann. Rpt. 1899, p. 104. 1900.

[3] Woods, C. D., and Merrill, L. H. Studies on the digestibility and nutritive value of bread at the Maine agricultural experiment station, 1899–1903. U. S. Dept. Agr. Office Exp. Stas. Bul. 143, p. 33. 1904.

[4] Grindley, H. S., Mojonnier, Timothy, and Porter, H. C. Studies of the effect of different methods of cooking upon the thoroughness and ease of digestion of meat at the University of Illinois. U. S. Dept. Agr. Office Exp. Stas. Bul. 193, 100 p. 1907.

# SOME PROPERTIES OF THE VIRUS OF THE MOSAIC DISEASE OF TOBACCO

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## INTRODUCTION

Several theories have been advanced to explain the physiological origin of the mosaic disease of tobacco (*Nicotiana tabacum.*) Independently, Woods (20) [1] and Heintzel (10) came to the conclusion that oxidizing enzyms are responsible for the disease. Hunger (11) did not accept the enzymic theory of the mosaic disease but considered that unfavorable conditions of growth produced specific toxins within the plant which led to the appearance of the disease. The writer (1) has secured data which do not lend support to the physiological origin of the disease, but indicate that it is dependent upon specific infection.

Further studies of the properties of the expressed sap of mosaic plants, termed the "virus" of the disease, have thrown considerable light on the nature of the infective principle and its relation to some of the enzymic properties of the sap of diseased plants.

Woods (20) and other workers following him have attributed the origin of the mosaic disease to oxidases and peroxidases existing normally in healthy tobacco plants. Since it is a question of fundamental importance to determine whether or not such enzyms are the primary cause of the disease, their relation to infection has been more fully investigated. All data at hand indicate that infection does not depend upon the presence of oxidases or peroxidases, but upon an infective principle which is not a normal constituent of the sap of healthy plants. These conclusions rest upon the fact that methods have been found by which the infective principle may be separated from the oxidases and peroxidases present in the sap of mosaic plants, as shown in the experimental work.

## FILTRATION EXPERIMENTS WITH THE VIRUS OF THE DISEASE

### FILTRATION THROUGH THE LIVINGSTONE ATMOMETER POROUS CUP

Earlier investigators have shown that the virus of the mosaic disease of tobacco passes through the Berkefeld (normal) filter without losing its infectious properties. The writer's experiments substantiate these results, as shown in Table I, although there is strong indication that the virus becomes attenuated and is less infectious when filtered in this

[1] Reference is made by number to "Literature cited," p. 673–674.

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way. The writer has been unable to obtain the finer pored Berkefeld or Pasteur-Chamberland bougies of the same type because of the European war. By using the Livingstone atmometer porous cup, however, a method of filtration has been devised which has given very interesting results. The construction of this apparatus is shown in Plate XCI. The extracted sap is first filtered through filter paper to remove all suspended material. The clear dark-amber solution is then filtered through the porous cup under reduced pressure (approximately 3 inches of mercury). After passing through the atmometer, the virus has completely lost its infectious properties, yet an intense peroxidase reaction is given with guaiac and hydrogen peroxid.[1]

TABLE I.—*Infectivity of the mosaic virus after it has been filtered through the Livingstone atmometer porous cup in 1915, 10 Connecticut Broadleaf plants having been used in each test*

| Virus used. | Enzymic reactions before treatment. | Treatment. | Enzymic reactions after treatment. | Date of inoculation. | Result. |
|---|---|---|---|---|---|
| Virus $X^{16}$..... | Intense peroxidase, intense catalase. | Untreated..... | Intense peroxidase, intense catalase. | Nov. 9 | 8 mosaic. |
| Do..... | .....do..... | Filtered through paper only. | .....do..... | ...do.... | Do. |
| Do..... | .....do..... | Filtered through atmometer. | .....do..... | ...do.... | All healthy. |
| Tap water (control). | ..... | ..... | ..... | ...do.... | Do. |
| Virus $X^{23}$..... | Intense peroxidase, weak catalase. | Filtered through paper only. | Intense peroxidase, weak catalase. | Nov. 24 | 4 mosaic. |
| Virus $X^{23}$, portion A. | .....do..... | Filtered through atmometer, taken after filtering 2 hours. | Intense peroxidase | ...do.... | All healthy. |
| Virus $X^{23}$, portion B. | .....do..... | Filtered through atmometer, taken after filtering 1 hour. | .....do..... | ...do.... | Do. |
| Virus $X^{23}$, portion C. | .....do..... | .....do..... | .....do..... | ...do.... | Do. |
| Virus $X^{23}$, portion D. | .....do..... | .....do..... | .....do..... | ...do.... | Do. |
| Tap water (control). | ..... | ..... | ..... | ...do.... | Do. |

## FILTRATION THROUGH POWDERED TALC

Numerous experiments have shown that the infective principle of the mosaic disease of tobacco may be completely removed by filtering the virus through powdered talc.

In these experiments (Table II) Hirsch's porcelain funnel, having a diameter of 9 to 10 cm. and furnished with a stationary perforated disk, was used. A disk of hard filter paper was placed over this disk to retain

[1] Woods used the guaiac and guaiac hydrogen-peroxid tests giving the blue coloration for the determination of oxidases and peroxidases in the extracted sap of tobacco plants. Since the oxidase theory as expressed by Woods was based upon results secured with these tests, the same tests were used in the writer's experiments. The terms "intense," "strong," "weak," etc., have been used to designate the relative intensity of the blue coloration. An "intense" peroxidase reaction is one giving at once an intense indigo blue. The term "strong" indicates that the blue coloration is not as deep, and appears more slowly. The term "weak" denotes a light-blue coloration.

the talc, which was mixed with water and poured upon the paper. This filter must be very carefully made, as bubbles and cracks which may form as a result of shrinkage due to drying during the process of making render the results unreliable. Filtration was accomplished by means of a reduced pressure of approximately 3 inches of mercury.

TABLE II.—*Infectivity of mosaic virus after having been filtered through different thicknesses of powdered talc, U. S. P., in 1915*

| Virus used. | Peroxidase reactions before filtering. | Material used for inoculation. | Peroxidase reactions after filtering. | Date of inoculation. | Number of plants inoculated. | Results. |
|---|---|---|---|---|---|---|
| $X^{100}$ | Intense | Filtrate from 1-inch talc | Intense | Mar. 6 | 20 | All healthy. |
| Do | ...do | Residue on surface of above talc | ...do | ...do | 10 | 7 mosaic. |
| Tap water (control). | | | | ...do | 10 | All healthy. |
| $X^{100}$ | Intense | Filtrate from 1-inch talc | Intense | Mar. 21 | 20 | Do. |
| Do | ...do | Residue on surface of above talc | ...do | ...do | 10 | 8 mosaic. |
| Tap water (control). | | | | ...do | 10 | All healthy. |
| $X^{99}$ | Intense | First portion of filtrate from 1¾-inch talc; color light amber. | None | Apr. 29 | 10 | Do. |
| Do | ...do | Second portion of above filtrate 2 hours later; color darker. | ...do | ...do | 20 | Do. |
| Do | ...do | Residue on surface of above talc | Intense | ...do | 20 | 19 mosaic. |
| $X^{97}$ | ...do | Filtrate from ⅞-inch talc | Strong | ...do | 10 | All healthy. |
| Do | ...do | ...do | ...do | ...do | 10 | Do. |
| Do | ...do | Filtrate from 2-inch talc | ...do | ...do | 10 | Do. |
| Tap water (control). | | | | ...do | 10 | Do. |
| $X^{96}$ | Intense | First portion of filtrate from 1½ inch talc; color light. | None | Apr. 30 | 10 | Do. |
| Do | ...do | Second portion of above filtrate; color very dark. | Intense | ...do | 10 | Do. |
| Do | ...do | Residue on surface of above talc | ...do | ...do | 10 | 10 mosaic. |
| $X^{98}$ | ...do | First portion of filtrate from 1½-inch talc; color light. | None | ...do | 10 | All healthy. |
| Do | ...do | Residue on surface of above talc | Strong | ...do | 20 | 9 mosaic. |
| $X^{100}$ | ...do | Filtrate from 1-inch talc | ...do | May 4 | 10 | All healthy. |
| Do | ...do | Residue on surface of above talc | ...do | ...do | 10 | 8 mosaic. |

Experiments (Table III) have shown that thick layers of talc, by adsorption, remove all the peroxidase from the pure virus. If no peroxidase reactions are shown, or if these reactions have been appreciably weakened, such filtrates have always lost their infectious properties. By reducing the amounts of talc, however, the peroxidase content may be increased until limits are reached beyond which the infective principle also passes into the filtrates. In some of the writer's filtration tests the first portions of the filtrate, giving intense peroxidase reactions, possessed no infectious properties, while the last portions contained the infectious principle of the disease. By using known quantities of powdered talc and constant quantities of different concentrations of virus, it is readily shown that the peroxidase content of the filtrates is not definitely related to infectivity. The Hirsch porcelain funnel was used as in preceding talc filtration tests. The virus was first filtered through paper to remove suspended material. All dilutions were made with distilled water. Filtration was accomplished by means of a reduced pressure of approximately 3 inches of mercury.

TABLE III.—*Infectivity of mosaic virus after filtering constant quantities of different concentrations through weighed amounts of powdered talc, U. S. P.*

| Strength of virus after dilution with distilled water. | Peroxidase reactions before filtering. | Quantity of talc. | Time required to filter 50 c. c. of solution. | Peroxidase reactions after filtering. | Number of plants inoculated. | Results. |
|---|---|---|---|---|---|---|
| | | *Gm.* | *Minutes.* | | | |
| 50 c. c. of undiluted virus | Intense | (a) | ..... | Intense | 20 | 15 mosaic. |
| Do | do | 36 | 30 | Weak | 10 | All healthy. |
| Do | do | 18 | 20 | Intense | 10 | Do. |
| 50 c. c. of 60 per cent virus | do | 18 | 9 | Strong | 10 | Do. |
| 50 c. c. of 40 per cent virus | do | (a) | ..... | do | 10 | 9 mosaic. |
| Do | Strong | 18 | 6 | None | 10 | All healthy. |
| 50 c. c. of 20 per cent virus | do | 18 | (b) | do | 10 | Do. |
| 50 c. c. of 4 per cent virus | do | 18 | do | do | 10 | Do. |
| 50 c. c. of 56 per cent virus | Intense | 9 | 9 | Strong | 10 | Do. |
| 50 c. c. of 24 per cent virus | Strong | 9 | (b) | Weak | 10 | Do. |
| 50 c. c. of 20 per cent virus | do | 9 | 5 | Very weak | 10 | Do. |
| 50 c. c. of 4 per cent virus | do | 9 | 4 | None | 10 | Do. |
| 50 c. c. of undiluted virus | Intense | 9 | 15 | Intense | 10 | Do. |
| 50 c. c. of 10 per cent virus | Strong | 4.5 | 5 | Weak | 10 | Do. |
| 50 c. c. of 4 per cent virus | do | 4.5 | 5 | None | 10 | Do. |
| 50 c. c. of 5 per cent virus | do | 2.3 | 2 | Good | 10 | Do. |
| 50 c. c. of 3 per cent virus | do | 2.3 | 1¾ | None | 10 | Do. |
| 50 c. c. of 2 per cent virus | Good | 1.1 | 1½ | do | 10 | Do. |

[a] Paper only. [b] Not timed.

These results indicate that the infective agents producing the mosaic disease are readily arrested by means of the talc filter. Likewise, it is shown that filtered solutions giving intense peroxidase reactions are no longer infectious.

## PRECIPITATION OF THE VIRUS WITH ETHYL ALCOHOL

Experiments have shown that the infective properties of the mosaic disease are quickly destroyed by the higher strengths of ethyl alcohol. Although a strength of 80 per cent appears to destroy the infective properties of the virus in half an hour, the peroxidase continues to give strong reactions with guaiac and hydrogen peroxid. In various experiments the enzyms have been precipitated in solutions of virus of sufficient alcoholic strength to destroy its infective properties. The virus was first passed through filter paper to remove all material in suspension. This gave a clear, dark, wine-colored solution, which was then made up to different alcoholic strengths with absolute alcohol.

In the first test virus $X^{16}$, giving intense peroxidase and catalase reactions, was used. On November 1, 1915, 200 c. c. of this virus were made up to a 75 per cent alcoholic strength with absolute alcohol. On November 2 the solution was filtered and the precipitate air dried to remove the alcohol. On November 3 the residue remaining was taken up with 50 c. c. of distilled water. Of the filtrate, 750 c. c. were then evaporated to dryness at room temperature, from November 2 to November 4. This filtrate contained neither peroxidase nor catalase. After evaporation, the amber-colored residue, which is readily soluble, was taken up with distilled water.

In a second test the highly infectious virus $X^{20}$, giving intense peroxidase reactions but no reaction for catalase, was used, and 400 c. c. of this virus

were made up to an alcoholic strength of 80 per cent with absolute alcohol. This solution was prepared on November 6, 1915, and allowed to stand until November 8, when the precipitate was collected by filtration and evaporated to dryness at room temperatures. This residue was taken up with 100 c. c. of distilled water. The original filtrate was also tested for peroxidase and likewise by inoculation.

Since earlier experiments have shown that the infective principle is not destroyed in alcoholic strengths of 45 to 50 per cent for several days, precipitation tests were also made with these strengths. Of virus $X^{20}$, used in the preceding test, 160 c. c. were made up to a 50 per cent alcoholic solution with absolute alcohol on November 6, 1915. A portion of the supernatant, clear solution was then siphoned off very carefully without disturbing the heavy, flocculent precipitate below. The precipitate was then collected on filter paper and freed from alcohol at room temperatures on November 8 to November 10. This residue was then taken up with 100 c. c. of distilled water (Table IV).

TABLE IV.—*Infectivity of mosaic virus after having been precipitated in 75, 80, 50, and 45 per cent alcoholic solutions*

| Virus used. | Alcoholic strengths. | Treatment. | Enzymic reactions after treatment. | Number of plants inoculated. | Result. |
|---|---|---|---|---|---|
| | *Per cent.* | | | | |
| $X^{16}$..... | 75 | Precipitate evaporated dry and taken up with 50 c. c. of water. | Intense peroxidase, intense catalase. | 10 | All healthy. |
| Do..... | 75 | Filtrate from above evaporated dry and taken up with water. | No peroxidase, no catalase. | 10 | Do. |
| Do..... | ..... | Original, untreated virus..... | Intense peroxidase, intense catalase. | 20 | 17 mosaic. |
| Tap water only (control). | ..... | ..... | ..... | 10 | All healthy. |
| $X^{20}$..... | 80 | Precipitate evaporated dry and taken up with 100 c. c. of water. | Intense peroxidase | 10 | Do. |
| Do..... | 80 | Filtrate from above not evaporated.. | No peroxidase..... | 10 | Do. |
| Do..... | ..... | 35 c. c. original virus evaporated dry and taken up with 20 c. c. of water. | Intense peroxidase, no catalase. | 10 | 5 mosaic. |
| Tap water only (control). | ..... | ..... | ..... | 10 | All healthy. |
| $X^{23}$..... | 50 | Precipitate evaporated dry and taken up with 100 c. c. of water. | Strong peroxidase. | 10 | 9 mosaic. |
| Do..... | 50 | Supernatant solution siphoned off from above precipitate and not evaporated. | .....do..... | 10 | All healthy. |
| Do..... | 45 | Precipitate not filtered or evaporated. | Intense peroxidase | 10 | 9 mosaic. |
| Do..... | 45 | Second portion of unfiltered precipitate, 15 c. c. diluted with 15 c. c. of water. | .....do..... | 10 | 10 mosaic. |
| Do..... | 45 | 200 c. c. of precipitate evaporated dry and taken up with 150 c. c. of water. | .....do..... | 10 | Do. |
| Do..... | 45 | Supernatant solution siphoned off, but not filtered or evaporated. | Strong peroxidase. | 10 | All healthy. |
| Do..... | 45 | Supernatant solution siphoned off and filtered through paper only. | .....do..... | 10 | Do. |
| Do..... | 45 | 15 c. c. of unfiltered supernatant solution diluted with 15 c. c. of water. | .....do..... | 10 | Do. |
| Do..... | 45 | 1,000 c. c. of filtered, supernatant solution evaporated dry and taken up with 400 c. c. of water. | .....do..... | 10 | Do. |
| Do..... | ..... | Original virus untreated, but diluted to 1 part of virus in 500 parts of water. | No peroxidase..... | 10 | 8 mosaic. |
| Tap water only (control). | ..... | ..... | ..... | 10 | All healthy. |

In the next experiment a 45 per cent alcoholic solution of virus was made up with virus $X^{23}$ as follows: 825 c. c. of this virus, which had been previously filtered through filter paper, were shaken with 675 c. c. of absolute alcohol on January 14, 1916. On January 15 the clear, supernatant solution was siphoned off. A portion of this was filtered through hard filter paper and a second portion was left unfiltered. Of the unfiltered portion 15 c. c. were also diluted with 15 c. c. of distilled water. On January 15, 1,000 c. c. of the supernatant solution which had been filtered through paper were set aside in a large, shallow dish to evaporate. On January 20 the dry residue was taken up with 400 c. c. of distilled water, which gave a somewhat stronger concentration than the original virus. (See Table IV.)

After decanting off as much of the supernatant solution as possible, the heavy, semiliquid precipitate, or sludge, was treated as follows: A portion was left unfiltered; a second portion was diluted by adding 15 c. c. of distilled water to 15 c. c. of the sludge. In addition to this, 200 c. c. of the sludge were placed in a beaker to evaporate to dryness at room temperatures on January 15. The dry residue was taken up with 150 c. c. of distilled water on January 17. Inoculation tests were now made with the virus after undergoing the various treatments outlined above in connection with precipitations with ethyl alcohol.

From Table IV it will be seen that the infective principle of the virus has been completely destroyed in the 75 per cent and 80 per cent alcoholic solutions, although the precipitates continued to give intense reactions for peroxidase. In these strengths precipitation of the peroxidase was complete, as the supernatant solutions gave no reaction for this enzym.

In the 45 per cent and 50 per cent alcoholic solutions, the infective principle was not appreciably injured. The infective agent, however, appears to have been carried down with the heavy, flocculent precipitates, leaving the supernatant solutions free from infective properties. Owing to the fact that the peroxidase remained in solution, the supernatant solutions continued to give strong peroxidase reactions. According to Chodat and Bach (7), the oxygenase in the sap of a species of Lactarius could be largely precipitated by 40 per cent alcohol, while the peroxidase remained in solution.

The writer's experiments indicate that concentrated solutions of peroxidase precipitated by strong alcohol from the sap of mosaic plants will not produce infection in healthy plants. Furthermore, the writer has carried out successive re-solutions in water and re-precipitations with alcohol in order to obtain purer solutions of peroxidase. Such solutions, however, have never produced infection, although giving intense reactions for peroxidase and in some instances for catalase.

## TREATMENT OF THE VIRUS WITH HYDROGEN PEROXID

Experiments have shown that certain quantities of hydrogen peroxid (U. S. P., 3.10 per cent) may be added to the virus of the mosaic disease without destroying its infectious properties. By treating the virus with different quantities of hydrogen peroxid, it is possible to find concentrations which destroy the peroxidase and at the same time leave little or no free hydrogen peroxid in the solution. Schönbein (19, p. 474) and likewise Bach and Chodat (2, p. 603) have observed that while peroxidase activates small amounts of hydrogen peroxid, large amounts of hydrogen peroxid destroy the peroxidase (Table V).

These results show that hydrogen peroxid may destroy the peroxidase in the virus without destroying its infectious properties. Although such solutions no longer give peroxidase reactions, they may retain their infectious properties for a long time. If the quantity of hydrogen peroxid is considerably increased beyond that concentration which is sufficient to destroy all the peroxidase, hydrogen peroxid remains in excess in the solution and the virus sooner or later loses its property of infection.

Chodat (6, p. 642–645) and other investigators have shown the definite relations existing between peroxidase, hydrogen peroxid, and the oxidation products. It has been shown that for constant quantities of peroxidase, the oxidation products increase directly with the amount of hydrogen peroxid present, within certain limits, until all the peroxidase is combined or used up.

The quantity of hydrogen peroxid required to destroy the peroxidase varies greatly, depending upon the composition of the virus. If the virus evolves little or no oxygen upon the addition of hydrogen peroxid, a very small quantity of this reagent destroys the peroxidase.

From Table V it will be seen that a very small quantity of hydrogen peroxid (3.1 per cent, U. S. P.) destroyed the peroxidase in virus $X^{20}$. As the quantities of hydrogen peroxid were increased, a point was reached where the excess was sufficient to kill the infective principle of the virus. If a considerable excess is present in solutions of virus for any length of time, such solutions lose their green or brown color and become pale or almost as clear as water in some instances. With the addition of 2 c. c. of hydrogen peroxid to 23 c. c. of virus $X^{20}$, a small excess of hydrogen peroxid was noticeable for several days, but this later disappeared. It has been observed by Bach and Chodat (3, p. 173) that if a mixture of peroxidase and hydrogen peroxid is allowed to stand for some time, both disappear from the solution by mutual interaction and destruction.

TABLE V.—*Effect of hydrogen peroxid upon the infectivity of virus secured from different sources in 1915, 10 plants having been used in each test*

| Virus used. | Date extracted. | Enzymic reactions before treatment. | Treatment. | Enzymic reactions after treatment. | Date inoculated. | Result. |
|---|---|---|---|---|---|---|
| $X^{8}$ | | Strong peroxidase | 15 c. c. virus+5 c. c. $H_2O_2$, prepared Jan. 2. | No peroxidase, Jan. 5 | Jan. 5 | 8 mosaic. |
| | | | | No peroxidase, May 29 | May 29 | 10 mosaic. |
| Do | | do | 17 c. c. virus+3 c. c. $H_2O_2$, prepared Jan. 2. | Fair peroxidase, Jan. 5 | Jan. 5 | Do. |
| | | | | Weak peroxidase, Jan. 12 | | |
| | | | | No peroxidase, May 29 | May 29 | 3 mosaic. |
| Do | | do | 18 c. c. virus+2 c. c. $H_2O_2$, prepared Jan. 2. | Good peroxidase, Jan. 5 | Jan. 5 | 8 mosaic. |
| Do | | do | 19.5 c.c. virus+0.5 c. c. $H_2O_2$, prepared Jan. 2. | Strong peroxidase, Jan. 5 | do | 7 mosaic. |
| Do | | do | Untreated | do | do | 10 mosaic. |
| Tap water (control) | | | | | do | All healthy. |
| $X^{83}$ | July 12 | Intense peroxidase | 5 c. c. virus+15 c. c. $H_2O_2$, prepared July 13. | No peroxidase, July 17 | Aug. 21 | 8 mosaic. |
| | | | | No peroxidase, Aug. 21 | | |
| Do | do | do | 8 c. c. virus+12 c. c. $H_2O_2$, prepared July 13. | No peroxidase, July 17 | do | Do. |
| | | | | No peroxidase, Aug. 21 | | |
| Do | do | do | 10 c. c. virus+10 c. c. $H_2O_2$, prepared July 13. | No peroxidase, July 17 | do | Do. |
| | | | | No peroxidase, Aug. 21 | | |
| Do | do | do | 12 c. c. virus+8 c. c. $H_2O_2$, prepared July 13. | No peroxidase, July 17 | do | 1 mosaic. |
| | | | | No peroxidase, Aug. 21 | | |
| Do | do | do | 15 c. c. virus+5 c. c. $H_2O_2$, prepared July 13. | Trace peroxidase, July 17 | do | 4 mosaic. |
| | | | | No peroxidase, Aug. 21 | | |
| Do | do | do | 16 c. c. virus+4 c. c. $H_2O_2$, prepared July 13. | Slight peroxidase, July 17 | do | 7 mosaic. |
| | | | | No peroxidase, Aug. 21 | | |
| Do | do | do | 18 c. c. virus+2 c. c. $H_2O_2$, prepared July 13. | Strong peroxidase, July 17 | do | 4 mosaic. |
| | | | | Strong peroxidase, Aug. 21 | | |
| Do | do | do | Untreated | Intense peroxidase, Aug. 21 | do | Do. |
| Tap water (control) | | | | | do | All healthy. |
| $X^{96}$ | Mar. 28 | Intense peroxidase, intense catalase. | 5 c. c. virus+15 c. c. $H_2O_2$, prepared July 1. | No peroxidase, July 10 | Aug. 23 | Do. |
| | | | | No peroxidase, Aug. 23, but $H_2O_2$ in excess | | |
| Do | do | do | 10 c. c. virus+10 c. c. $H_2O_2$, prepared July 1. | No peroxidase, July 6 | do | 10 mosaic. |
| | | | | No peroxidase, Aug. 23 | | |
| | | | | No $H_2O_2$ present on Aug. 23 | | |
| Do | do | do | 15 c. c. virus+5 c. c. $H_2O_2$, prepared July 1. | Strong peroxidase, July 10 | do | 7 mosaic. |
| | | | | Good peroxidase, Aug. 23 | | |
| Do | do | do | Untreated | Intense peroxidase, Aug. 23 | do | 5 mosaic. |
| Tap water (control) | | | | | do | All healthy. |
| $X^{81}$ | July 8 | Intense peroxidase, strong catalase. | 10 c. c. virus+15 c. c. $H_2O_2$, prepared July 8. | No peroxidase, but $H_2O_2$ in excess Aug. 25 | Aug. 25 | Do. |
| Do | do | do | 10 c. c. virus+10 c. c. $H_2O_2$, prepared July 8. | No peroxidase, no $H_2O_2$. July 10 | do | 9 mosaic. |
| | | | | No peroxidase, no $H_2O_2$. Aug. 25 | | |
| | | | | No peroxidase, no $H_2O_2$. Oct. 23 | Oct. 23 | 4 mosaic. |
| Do | do | do | 15 c. c. virus+5 c. c. $H_2O_2$, prepared July 8. | No peroxidase, no $H_2O_2$. July 10 | Aug. 25 | 7 mosaic. |
| | | | | No peroxidase, no $H_2O_2$. Aug. 25 | | |
| Do | do | do | 17 c. c. virus + 3 c. c. $H_2O_2$ prepared July 8. | No peroxidase, no $H_2O_2$. July 10 | | |
| | | | | No peroxidase, no $H_2O_2$. Aug. 25 | Aug. 25 | 8 mosaic. |
| | | | | No peroxidase, no $H_2O_2$. Oct. 23 | Oct. 23 | 4 mosaic. |

| | | | | | | |
|---|---|---|---|---|---|---|
| Do | ...do... | .....do..... | Untreated | Intense peroxidase, Aug. 25<br>Strong catalase, Aug. 25 | Aug. 25 | 9 mosaic. |
| Tap water (control) | | | | | ...do... | All healthy. |
| $X^{20}$ | Nov. 1 | Intense peroxidase, no catalase. | 24 c. c. virus+1 c. c. $H_2O_2$ prepared Nov. 17. | Good peroxidase, Nov. 18<br>Very weak peroxidase, Nov. 22<br>No peroxidase, Nov. 27<br>No peroxidase, Dec. 20<br>No $H_2O_2$, Dec. 20 | Dec. 21 | 7 mosaic. |
| Do | ...do... | .....do..... | 23 c.c. virus+2 c c. $H_2O_2$ prepared Nov. 17. | Weak peroxidase, Nov. 18<br>No peroxidase, Nov. 22<br>$H_2O_2$ present, Nov. 22<br>No $H_2O_2$ present, Dec. 20 | ..do... | 5 mosaic. |
| Do | ...do... | .....do..... | 22 c. c. virus+3 c. c. $H_2O_2$ prepared Nov. 17. | No peroxidase, Nov. 18<br>No peroxidase, Dec. 20<br>$H_2O_2$ in excess, Dec. 20 | ..do... | 4 mosaic. |
| Do | ...do... | .....do..... | 21 c. c. virus+4 c. c. $H_2O_2$ prepared Nov. 17. | No peroxidase, Nov. 18<br>No peroxidase, Dec. 20<br>$H_2O_2$ in excess, Dec. 20 | do... | All healthy. |
| Do | ...do... | .....do..... | 20 c. c. virus+5 c. c. $H_2O_2$ prepared Nov. 17. | No peroxidase, Nov. 18<br>No peroxidase, Dec. 20<br>Much excess $H_2O_2$, Dec. 20 | do... | Do. |
| Do | ...do... | .....do..... | 19 c. c. virus+6 c. c. $H_2O_2$ prepared Nov. 17. | No peroxidase, Nov. 18<br>No peroxidase, Dec. 20<br>Much excess $H_2O_2$, Dec. 20 | do... | Do. |
| Do | ...do... | .....do..... | Untreated | Intense peroxidase | ...do... | 8 mosaic. |
| Tap water (control) | | | | | ...do... | All healthy. |

## TREATMENT OF THE VIRUS WITH FORMALDEHYDE

Although formaldehyde destroys the infective principle of the virus in certain concentrations, peroxidase is not appreciably injured for a considerable time at much greater concentrations. Loew (15, p. 20) has shown that the peroxidase of tobacco is unaltered in a 5 per cent solution after 48 hours. It appears that some oxidases also are very resistant to formaldehyde. Kastle (13) found that the oxidase of the mushroom *Lepiota americana* is not destroyed by a 40 per cent formic-aldehyde solution which is allowed to act for several days. In the writer's test (Table VI), the peroxidase of tobacco was not appreciably changed in 1 per cent solutions of formaldehyde after standing 30 days. In these tests a 37 per cent U. S. P. solution of formaldehyde was used. All concentrations were made on the assumption that 2.5 c. c. of this solution contained about 1 gm. of formaldehyde. The virus was filtered through paper to remove all suspended material. To subject the virus to a certain strength of formaldehyde, a water solution of formaldehyde just twice as strong as desired for the virus was made up. Equal parts of this solution and the virus were then mixed, thus bringing the formaldehyde strength down to that required for the virus. In this way the virus was uniformly diluted to one-half its original strength in all concentrations of formaldehyde.

TABLE VI.—*Infectivity of the mosaic virus after 31 days' treatment with formaldehyde in 1915, 10 plants having been used in each test*

| Virus used. | Enzymic reactions before treatment. | Strength of formaldehyde in the virus solutions. | Date prepared. | Enzymic reactions after treatment. | Date inoculated. | Result. |
|---|---|---|---|---|---|---|
| $X^{20}$..... | Intense peroxidase. | 1 : 100..... | Nov. 17 | Intense peroxidase, Dec. 17. | Dec. 18 | All healthy. |
| Do..... | .....do..... | 1 : 200..... | ...do..... | .....do..... | ...do..... | Do. |
| Do..... | .....do..... | 1 : 400..... | ...do..... | .....do..... | ...do..... | Do. |
| Do..... | .....do..... | 1 : 600..... | ...do..... | .....do..... | ...do..... | Do. |
| Do..... | .....do..... | 1 : 800..... | ...do..... | .....do..... | ...do..... | 1 mosaic. |
| Do..... | .....do..... | 1 : 1,000..... | ...do..... | .....do..... | ...do..... | Do. |
| Do..... | .....do..... | 1 : 1,200..... | ...do..... | .....do..... | ...do..... | 7 mosaic. |
| Do..... | .....do..... | 1 : 1,500..... | ...do..... | .....do..... | ...do..... | 5 mosaic. |
| Do..... | .....do..... | Untreated..... | ...do..... | .....do..... | ...do..... | 9 mosaic. |
| Tap water (control). | ..... | ..... | ..... | ..... | ...do..... | All healthy. |

Experiments carried out in 1914 with unfiltered virus treated with the same strengths of formaldehyde and tested 32 days later gave practically the same results. In these tests the virus still retained its infectious properties in that solution which contained 1 part of formaldehyde in 1,000 parts of virus solution. All stronger solutions had lost the power to produce infection.

## TREATMENT OF DRIED MOSAIC MATERIAL WITH ETHER, CHLOROFORM, AND OTHER SOLVENTS

In the following experiments dried and ground mosaic material, designated as $X^{13}$, was used. The original green leaves were harvested on August 31, 1915, and dried in the air. For each solvent the procedure was as follows: Ten gm. of air-dry material were extracted with 70 c. c. of extractive for two days. This solution was then filtered through paper and 35 c. c. of the filtered solution were set aside in a small beaker to evaporate at room temperatures. The residue left after evaporation was brought into 5 c. c. of distilled water and used for inoculation. The original residue, $X^{13}$, left after filtering off the solvent, was then thoroughly dried at room temperatures and macerated with 50 c. c. of distilled water in a mortar. Ten c. c. of the extract were used for inoculation. In this way the infective properties of the extract and of the original material from which this extract was obtained could be compared.

The process was somewhat different with glycerin, as this is not readily evaporated. After extracting 10 gm. of the dry material for two days the glycerin extract was pressed out and filtered through hard paper under reduced atmospheric pressure. Of the filtered solution 40 c. c. were then made up to 800 c. c. with distilled water, giving a 5 per cent glycerin solution, which will produce no injury when inoculated into tobacco plants. After filtering off the glycerin extract the original $X^{13}$ residue was then subjected to pressure to remove as much glycerin as possible and was then macerated in a mortar with 50 c. c. of distilled water. The results obtained for each solvent are shown in Table VII.

TABLE VII.—*Effect of digestion with ether, chloroform, and other solvents upon infectivity of mosaic material $X^{13}$ dried at room temperatures in 1915, 10 plants having been used in each test*

| Solvent used. | Period of extraction. | Time required for extract to evaporate. | Date taken up with water. | Peroxidase reaction after treatment. | Results of inoculating plants Oct. 19, 1915. |
|---|---|---|---|---|---|
| Residue $X^{13}$ after digestion with ether. | Oct. 6 to 8... | Oct. 8 to 16.... | Oct. 16 | Very weak.. | 8 mosaic. |
| Ether extract of $X^{13}$..... | .....do..... | Oct. 8 to 12.... | Oct. 12 | None..... | All healthy. |
| Residue $X^{13}$ after digestion with chloroform. | .....do..... | Oct. 8 to 16.... | Oct. 16 | Very weak.. | 10 mosaic. |
| Chloroform extract of $X^{13}$..... | .....do..... | Oct. 8 to 12.... | Oct. 12 | None..... | All healthy. |
| Residue $X^{13}$ after digestion with carbon tetrachlorid. | .....do..... | Oct. 8 to 16.... | Oct. 16 | Very weak.. | 5 mosaic. |
| Carbon tetrachlorid extract of $X^{13}$. | .....do..... | Oct. 8 to 12.... | Oct. 12 | None..... | All healthy. |
| Residue $X^{13}$ after digestion with toluene. | .....do..... | Oct. 8 to 16.... | Oct. 16 | Very weak.. | 10 mosaic. |
| Toluene extract of $X^{13}$..... | .....do..... | Oct. 8 to 12.... | Oct. 12 | None..... | All healthy. |
| Residue $X^{13}$ after digestion with acetone. | .....do..... | Oct. 8 to 16.... | Oct. 16 | Very weak.. | 8 mosaic. |
| Acetone extract of $X^{13}$..... | .....do..... | Oct. 8 to 12.... | Oct. 12 | None..... | All healthy. |
| Residue $X^{13}$ after digestion with ethyl alcohol. | Oct. 8 to 10.. | Oct. 10 to 16... | Oct. 16 | Weak..... | Do. |
| Ethyl alcohol extract of $X^{13}$..... | .....do..... | Oct. 10 to 13... | Oct. 13 | None..... | Do. |
| Residue $X^{13}$ after digestion with methyl alcohol. | .....do..... | Oct. 10 to 16... | Oct. 16 | Very weak.. | Do. |
| Methyl alcohol extract of $X^{13}$..... | .....do..... | Oct. 10 to 15... | Oct. 15 | None..... | Do. |
| Residue $X^{13}$ after digestion with glycerin. | .....do..... | Not evaporated. | Oct. 16 | Very weak.. | 6 mosaic. |
| Glycerin extract of $X^{13}$..... | .....do..... | .....do..... | Oct. 11 | .....do..... | 10 mosaic. |
| Residue $X^{13}$ after digestion with water. | .....do..... | Oct. 10 to 16... | Oct. 16 | .....do..... | Do. |
| Water extract of $X^{13}$..... | .....do..... | Not evaporated. | Oct. 11 | Good..... | 8 mosaic. |
| Tap water (control)..... | ..... | ..... | ..... | ..... | All healthy. |

In the test with glycerin the material to which the glycerin had been added was subjected to maceration and pressure in order to obtain the extract. A later experiment would seem to indicate that if the glycerin extract is poured off without subjecting the residue to maceration or pressure the extract will contain little, if any, of the infectious principle. In this experiment 10 gm. of the same $X^{13}$ material were used. This material, however, was dried over sulphuric acid in a desiccator from November 8 to November 23, 1915. On November 23, 80 c. c. of glycerin were added and allowed to stand until November 27, when the dark-colored extract was merely poured off and filtered through hard paper under reduced atmospheric pressure. Solutions containing 8 and 20 per cent of the extract were made, distilled water being used to dilute the glycerin.

The dry material from which the glycerin extract had been poured off was now washed with 100 c. c. of distilled water. This was poured off and filtered through hard paper, and the material was again washed with 380 c. c. of distilled water. This solution was also poured off and filtered through hard paper. The original leaf material, which was now fairly free from glycerin, was macerated with 25 c. c. of distilled water. The results of testing the above solutions and material for peroxidase and infection are given in Table VIII.

TABLE VIII.—*Results of inoculations with dried material $X^{13}$ digested with glycerin in 1915, 10 plants having been used in each test*

| Material used. | Peroxidase reaction after treatment. | Date inoculated. | Result. |
|---|---|---|---|
| 8 per cent glycerin extract | No peroxidase. (Nov. 27 / Dec. 15) | Dec. 16 | 1 mosaic. |
| 20 per cent glycerin extract | do. | do. | All healthy. |
| 100 c. c. water solution (first washing) | do. | do. | 1 mosaic. |
| 380 c. c. water solution (second washing) | do. | do. | 4 mosaic. |
| Residue $X^{13}$ macerated with 25 c. c. $H_2O$ | Weak peroxidase. (Nov. 27 / Dec. 15) | do. | 10 mosaic. |
| Tap water only (control) | | do. | All healthy. |

In order to compare the results with dried mosaic material, green mosaic leaf material was also treated with ether, chloroform, and water. For each solvent 25 gm. of finely cut and macerated green mosaic material were used. The quantity of solvent used was about 100 gm.—that is, 140 c. c. of ether, 80 c. c. of chloroform, and 100 c. c. of distilled water. These solvents were added to the green material and shaken on October 20. On October 21 all the solution that could be poured off was then filtered through hard paper and set aside in beakers to evaporate in the air. Of the filtered ether solution 115 c. c., and of the chloroform solution 55 c. c., were obtained. After evaporation, the residues left from the ether and chloroform solutions were each placed in 5 c. c. of distilled water. The leaf material from which these solutions had been obtained

was again dried at room temperatures and macerated with 20 c. c. of distilled water. The results of inoculation experiments with this material are shown in Table IX.

TABLE IX.—*Infectivity of green mosaic leaf material after digestion with ether, chloroform, and water, October 20 and 21, 1915, 10 plants having been used in each test*

| Material used. | Time required for extract to evaporate. | Date taken up with water. | Peroxidase reaction after digestion. | Results of inoculating plants Oct. 25, 1915. |
|---|---|---|---|---|
| Green residue after digestion with ether. | Oct. 21 to 23..... | Oct. 23 | Intense peroxidase, Oct. 25.. | 10 mosaic. |
| Ether solution from above... | Oct. 21 to 22..... | Oct. 22 | Good peroxidase, Oct. 25.... | 6 mosaic. |
| Green residue after digestion with chloroform. | Oct. 21 to 23..... | Oct. 23 | Intense peroxidase, Oct. 25.. | 10 mosaic. |
| Chloroform solution from above. | .....do..... | .....do... | .....do..... | 9 mosaic. |
| Green residue after digestion with water. | .....do..... | .....do... | Good peroxidase, Oct. 25.... | 10 mosaic. |
| Water solution from above... | Not evaporated. | ..... | Intense peroxidase, Oct. 25.. | Do. |
| Tap water (control)..... | ..... | ..... | ..... | All healthy. |

From Table IX it is evident that the infective principle of the virus was not killed in the ether or chloroform solutions. From similar experiments Clinton (8, p. 415) believed that ether and chloroform could extract the virus from the green leaves to some extent without injury to its infectious properties.

However, from the fact that green crushed material contains a large amount of water, it is very probable that some of this water containing the infective principle passes into the ether or chloroform solutions. Such solutions would represent little more than mixtures of virus and ether, etc. Although the infective principle and likewise peroxidase appeared in the ether and chloroform solutions when green material was used, these did not appear in ether or chloroform extracts made with dry material.

The fact that the infective principle, or even enzyms, appeared in solutions obtained by adding ether, chloroform, toluene, etc., to green material does not justify the conclusion that such substances are soluble in these solvents. Kastle (13, p. 16), working with the oxidases of *Lepiota americana*, found that if toluene is added to portions of the fresh fungus, some of the oxidase passes into the toluene layer. He says:

> Whether the perfectly dry oxidase is soluble in toluene remains to be proved. It may be, of course, that it is the water which is dissolved in the toluene which really takes the oxidase into solution.

Various experiments have shown that the infective principle of the mosaic disease of tobacco is not readily destroyed by ether, chloroform, toluene, or carbon tetrachlorid. Although ether or chloroform vapors quickly kill the green leaf, the infective principle in a mosaic leaf killed in this way remains uninjured after several hours' treatment. Likewise

virus solutions to which several cubic centimeters of ether, chloroform, or toluene have been added did not lose their infectious properties after several months. Carbon tetrachlorid also appears to be quite as inert when added to virus solutions. In a test with this material, 3 c. c. were added to 22 c. c. of virus. The supernatant virus, when tested one month later, was quite as infectious as the untreated.

In other experiments the mosaic sap has been evaporated to dryness at room temperatures in beakers and the residue treated with ether for several days. Under such conditions, however, the residue is only slightly soluble in the ether and there remains a heavy, gummy, more or less impermeable mass. When the ether was evaporated and the residue again taken up with the original amount of water, the solution was still infectious.

Although Clinton (8, p. 415) states that the virus can be preserved for a long time by adding to it a small amount of toluene, the writer's experiments indicate that the virus will retain its infectious properties almost indefinitely without the addition of toluene. With no preservative whatever added, the bottled virus was highly infectious when tested from 12 to 15 months later, although putrefaction had taken place.

## TREATMENT OF VIRUS WITH PRECIPITATES OF HYDROXIDS OF ALUMINUM AND NICKEL

Precipitation of the virus of the mosaic disease of tobacco by alcohol in 45 and 50 per cent strengths indicates that the precipitate carries down the infective principle, leaving the supernatant solution without infectious properties. Similar precipitation experiments have been carried out, using aluminum sulphate and nickel sulphate in alkaline solutions of virus to obtain the insoluble hydroxids of these metals. In order to obtain approximately 1 gm. of aluminum hydroxid in the precipitate aluminum sulphate and sodium hydroxid were added according to the following equation:

$$Al_2(SO_4)_3.18H_2O+6NaOH=3\ Na_2SO_4+Al_2(OH)_6+18H_2O.$$
$$4.26\text{ gm.} \quad 1.53\text{ gm.} \quad 2.72\text{ gm.} \quad 1\text{ gm.}$$

The procedure was as follows: On December 1, 1915, 100 c. c. of virus $X^{23}$, which had been filtered through paper to obtain a clear solution, were made up to 1,000 c. c. with distilled water, thus diluting the virus but 10 times. First, 4.3 gm. of aluminum sulphate dissolved in a small quantity of water were added to the virus solution and shaken. Then 1.5 gm. of sodium hydroxid, dissolved in a small quantity of water, were added and the entire solution shaken and set aside. A very heavy flocculent precipitate of aluminum hydroxid was at once formed. This gradually settled, leaving the supernatant solution perfectly clear. On December 2 the solution was tested with litmus paper and gave a slightly

acid reaction. On this date the greater portion of the clear supernatant solution was carefully siphoned off. After pouring off as much of the remaining supernatant solution as possible, the semiliquid precipitate, or sludge, was bottled. The clear supernatant solution, as well as the sludge, gave intense peroxidase reactions.

As seen from the equation, the treatment of the original virus solution involves the formation of 2.7 gm. of the soluble salt (sodium sulphate) or 1 part in 370 of solution.

As a control to this there was prepared on December 1 a solution of virus of the same dilution as the original, containing 6 gm. of sodium sulphate per 1,000 c. c. of solution, or approximately 1 part of sodium sulphate in 303 parts of solution. It will be noted that this concentration is somewhat higher than that obtained in the reaction to produce 1 gm. of aluminum hydroxid.

The preparation of the nickel-sulphate solution, involving the formation of 2 gm. of nickel hydroxid, was carried out in the same manner as for the aluminum hydroxid. The results of inoculations made with the supernatant solutions and precipitates of aluminum hydroxid and nickel hydroxid are given in Table X.

TABLE X.—*Effect of aluminum hydroxid and nickel hydroxid upon the infectivity of mosaic virus, 10 plants having been used in each test. Material prepared on December 1, 1915*

| Material tested. | Enzymic reaction after treatment. | Result of inoculating plants. |
|---|---|---|
| Inoculations made on December 16, 1915: | | |
| Semifluid aluminum hydroxid precipitate | Intense peroxidase, Dec. 15, 1915. | 1 mosaic. |
| Do | do | 3 mosaic. |
| Supernatant solution from above precipitate | do | All healthy. |
| Semifluid nickel hydroxid precipitate | do | Do. |
| Supernatant solution from above precipitate | do | Do. |
| Sodium-sulphate-virus solution (1 part sodium sulphate in 303 parts of solution). | do | 10 mosaic. |
| Do | do | Do. |
| Original virus $X^{23}$ used in above tests, untreated | do | 4 mosaic. |
| Tap water (control) | | All healthy. |
| Inoculations made with above material on January 18, 1916: | | |
| Semifluid aluminum hydroxid precipitate | Intense peroxidase, Jan. 17, 1916. | 6 mosaic. |
| Supernatant solution from above precipitate | do | All healthy. |
| Semifluid nickel hydroxid precipitate | do | 1 mosaic. |
| Supernatant solution from above precipitate | do | All healthy. |
| Original virus $X^{23}$ used in above tests, untreated | do | 10 mosaic. |
| Tap water (control) | | All healthy. |

From the results of Table X it is quite evident that the infective principle of the virus was carried down with the aluminum hydroxid precipitate, leaving the supernatant solution free from infectious properties. Since the treatment with nickel sulphate appears to have destroyed the virus entirely, it is possible that nickel salts are more toxic to the infective principle than the salts of aluminum.

## EFFECT OF HEAT UPON THE VIRUS OF THE MOSAIC DISEASE

Several investigators have noted the effect of heat upon the virus of the mosaic disease. Mayer (17, p. 451) found that continued heating at 60° C. did not perceptibly change the infectivity of the virus, but that temperatures of 65° to 75° weakened it. Its infectious properties were completely destroyed when the virus was heated for several hours at 80°.

TABLE XI.—*Effect of heat upon the infectivity of undiluted solutions of mosaic virus heated without previous filtering, in 1915, 10 plants having been used in each test*

| Virus used. | Enzymic reaction before treatment. | Date and nature of treatment. | Enzymic reaction after treatment. | Date inoculated. | Results. |
|---|---|---|---|---|---|
| $X^{11}$.... | Intense peroxidas | Heated 15 minutes at 82° C. in test tube suspended in beaker of water, Nov. 23. | Good peroxidase, Nov. 24. | Nov. 24 | 10 mosaic. |
| Do.. | .....do..... | Boiled 1 minute in test tube, Nov. 23. | .....do..... | ...do..... | All healthy. |
| Do.. | .....do..... | Boiled 5 minutes in test tube, Nov. 23. | Weak peroxidase, Nov. 24. | ...do..... | Do. |
| Do.. | .....do..... | Boiled 10 minutes in test tube, Nov. 23. | .....do..... | ...do..... | Do. |
| Do.. | .....do..... | Unheated..... | Intense peroxidase, Nov. 24. | ...do..... | 8 mosaic. |
| X°..... | .....do..... | Heated 10 minutes at 85° C. in test tube suspended in beaker of water, Dec. 2. | Fair peroxidase, Dec. 3. | Dec. 3 | 9 mosaic. |
| Do.. | .....do..... | Heated 15 minutes at 85° C., Nov. 23; again heated 7 minutes at 88° C., Dec. 3. | No peroxidase, Dec. 4. | Dec. 4 | All healthy. |
| Do.. | .....do..... | Heated 10 minutes at 90° C. in test tube suspended in beaker of water, Dec. 2. | Weak peroxidase, Dec. 3. | Dec. 3 | Do. |
| Do.. | .....do..... | Heated 10 minutes at 95° C. in test tube suspended in beaker of water, Dec. 2. | Very weak peroxidase, Dec. 3. | ...do..... | Do. |
| Do.. | .....do..... | Heated 1 to 2 minutes at 100° C. in test tube suspended in beaker of water, Dec. 2. | Fair peroxidase, Dec. 3. | ...do..... | Do. |
| Do.. | .....do..... | Unheated..... | Intense peroxidase, Dec. 3. | Dec. 4 | 8 mosaic. |
| Control | Strong peroxidase. | Tap water and healthy juice, untreated. | Strong peroxidase, Dec. 4. | ...do..... | All healthy. |
| $X^{35}$..... | Intense peroxidase | Heated 5 minutes at 85° C. in test tube suspended in beaker of water, May 8. | Good peroxidase, May 8. | May 10 | 9 mosaic. |
| Do.. | .....do..... | Heated 5 minutes at 87° C. in test tube suspended in beaker of water, May 8. | .....do..... | ...do..... | 8 mosaic. |
| Do.. | .....do..... | Heated 5 minutes at 88° C. in test tube suspended in beaker of water, May 8. | .....do..... | ...do..... | Do. |
| Do.. | .....do..... | Heated 5 minutes at 90° C. in test tube suspended in beaker of water, May 8. | Fair peroxidase, May 8. | ...do..... | 1 mosaic. |
| Do.. | .....do..... | Heated 5 minutes at 91° C. in test tube suspended in beaker of water, May 8. | .....do..... | ...do..... | All healthy. |
| Do.. | .....do..... | Heated 5 minutes at 93° C. in test tube suspended in beaker of water, May 8. | .....do..... | ...do..... | Do. |
| Do.. | .....do..... | Heated 5 minutes at 95° C. in test tube suspended in beaker of water, May 8. | Very faint peroxidase, May 8. | ...do..... | Do. |
| Do.. | .....do..... | Unheated..... | Intense peroxidase, May 8. | ...do..... | 7 mosaic. |
| Control | Strong peroxidase. | Tap water and healthy juice, untreated. | Strong peroxidase, May 10. | ...do..... | All healthy. |

Iwanowski (12) and, likewise, Beijerinck (4) found that heating the virus to the boiling point destroyed its infectious properties.

According to Koning (14, p. 71–86), who heated the diluted virus in closed tubes, it remained infective when heated 10 minutes at 80°, 5 minutes at 90°, and 5 minutes at 100°.

Woods (20, p. 17–19) believed that the sap of mosaic plants remained infectious to some extent after it had been boiled, owing to the fact that peroxidase was regenerated in the solution.

The writer's experiments indicate that the infective principle of the virus is quickly and permanently destroyed at temperatures near the boiling point, although such solutions may again show good peroxidase reactions. In these tests, in which the test tube containing the virus was suspended in a beaker of heated water, the virus was kept at room temperatures until immersed. The test tube was immersed when the temperature in the beaker had begun to exceed the required point. Owing to the small quantities of virus used, the temperatures in all instances were very quickly brought up to the desired height.

The infective principle of the disease withstands much higher temperatures when the dried mosaic leaf material is subjected to dry heat. In the following experiments the air-dried mosaic leaves were finely ground and dried over sulphuric acid in a desiccator from October 8 to the date of heating. For each test 5 gm. of this powdered material were heated in an electric oven, then macerated and extracted with 25 c. c. of distilled water. The results shown in Table XII were obtained.

TABLE XII.—*Effect of heat upon the infectivity of dried mosaic leaf material in 1915, 10 plants having been used in each test*

| Date heated. | Period of heating. | Enzymic reaction after treatment. | Date inoculated. | Results. |
|---|---|---|---|---|
| Oct. 15 | ½ hour at 90° C. | Fair peroxidase, Oct. 22. | Oct. 23 | 10 mosaic. |
| Do. | ½ hour at 100° C. | Trace peroxidase, Oct. 22. | do. | Do. |
| Do. | One hour at 100° C. | do. | do. | 7 mosaic. |
| Do. | ½ hour at 110° C. | do. | do. | 9 mosaic. |
| Do. | ½ hour at 120° C. | No peroxidase, Oct. 22. | do. | 7 mosaic. |
| | Dry material, unheated. | Fair peroxidase, Oct. 22. | do. | 10 mosaic. |
| | Tap water only, unheated. | | do. | All healthy. |
| Nov. 5 | ½ hour at 140° C. | No peroxidase, Nov. 10. | Nov. 12 | Do. |
| Do. | ½ hour at 150° C. | do. | do. | Do. |

Heating experiments were again carried out, using the virus after it had been evaporated to dryness in small beakers. For each test 40 c. c. of undiluted and unfiltered virus $X^{16}$ were allowed to evaporate by exposure to the air on October 10, 1915. On November 4 the beakers containing the air-dried residues were heated in an electric oven. After being heated the residues were immediately taken up with 30 c. c. of distilled water.

TABLE XIII.—*Effect of heat upon the infectivity of mosaic virus which has been evaporated to dryness at room temperatures in 1915, 10 plants having been used in each test*

| Date heated. | Period of heating. | Enzymic reaction after treatment. | Date inoculated. | Results. |
|---|---|---|---|---|
| Nov. 4 | ½ hour at 100° C. | Fair peroxidase, Nov. 11<br>Trace catalase, Nov. 11 | Nov. 12 | All healthy. |
| Do. | ½ hour at 110° C. | No peroxidase, Nov. 11<br>No catalase, Nov. 11 | ..do.... | Do. |
| Do. | ½ hour at 120° C. | .....do..... | ...do.... | Do. |
| Do. | ½ hour at 130° C. | .....do..... | ...do.... | Do. |
| | Original virus evaporated air-dry, but not heated. | Weak peroxidase, Nov. 11<br>Intense catalase, Nov. 11 | ..do.... | 5 mosaic. |

From the data in Table XIII it is evident that the evaporated virus solution lost its infectious properties much more quickly than the dried and ground mosaic leaf material. Likewise, the peroxidase was somewhat more quickly destroyed in the evaporated material. Although the presence of small amounts of moisture in the air-dried residue of the evaporated virus may have hastened the destruction of the infective principle in this material, it is also possible that the infective principle is better able to withstand high temperatures when allowed to remain within the tissues of the leaf.

Although the virus with which Koning (14, p. 71–86) worked appears to have withstood temperatures as high as 100° C., the virus with which the writer worked is very quickly destroyed at temperatures above 90°. In some instances, however, the virus has been rendered noninfectious at a temperature 10 degrees lower than this. These results were obtained with the virus designated as $X^{16}$. This virus was extracted from tobacco plants on September 20, 1915, and bottled until used on October 18, 1915. Although the unheated virus was highly infectious, the infective principle was destroyed after heating for 5 minutes at 80°. It was also destroyed when heated for 2 minutes at 81°. Although the original, unheated virus gave intense reactions for catalase and peroxidase, the catalase was completely destroyed in these tests. Weak peroxidase reactions, however, were again shown the next day.

As Woods (20) has shown, there is frequently a return of peroxidase activity in solutions of virus that have been once heated. This activity does not appear to return immediately after cooling, but usually requires some hours for its return. Woods considered that the enzyn was destroyed in such solutions but a resistant zymogen again generated more of the peroxidase after cooling. On the other hand, Hasselbring and Alsberg (9) were led to believe from their experiments that a zymogen might not be present, but that the enzym was included and protected in the coagulum and subsequently leached out on standing.

By heating the virus several times at 85° C., the writer has been able in some instances to destroy completely the peroxidase present without destroying the infective principle. The highly infectious virus designated as X° and showing intense peroxidase reactions was treated as

follows: Heated 10 minutes at 85° on December 2, 1914. Cooled at once. A fair peroxidase reaction shown on December 3. Again heated 10 minutes at 85° on December 4, and cooled at once. Weak peroxidase reaction shown December 5. Again heated 10 minutes at 85° on December 5 and immediately cooled. Very weak peroxidase reaction was shown on December 16, when it was again heated for the fourth time for 15 minutes at 85°. When used for inoculation on December 28, no peroxidase reaction was shown. The virus was still highly infectious, however, and produced the mosaic disease in 9 out of 10 plants.

## EFFECT OF LOW TEMPERATURES UPON THE VIRUS

After having been frozen for periods varying from one to four hours at −12° C., the extracted sap of mosaic plants still retained its infectious properties unchanged. It likewise retained its original virulence after having been exposed outdoors during the entire winter of 1915 and allowed to freeze and thaw repeatedly. In recent experiments liquid air was used to freeze the virus, and a temperature of approximately −180° was reached. The results are given in Table XIV.

TABLE XIV.—*Effect upon infectivity of freezing fresh mosaic sap to −180° C. by means of liquid air in 1916, 10 plants having been used in each test*

| Material used. | Time exposed to liquid air. | Peroxidase reaction before freezing. | Peroxidase reaction after freezing. | Result of inoculating plants, Feb. 2, 1916. |
|---|---|---|---|---|
| | *Minutes.* | | | |
| Original virus, unfrozen | ..... | Intense, Feb. 1 | ..... | 10 mosaic. |
| Original virus, frozen Jan. 31 | 15 | .....do..... | Intense, Feb. 1 | Do. |
| Duplicate of above | 15 | .....do..... | .....do..... | Do. |
| Tap water and healthy sap, unfrozen | ..... | .....do..... | ..... | All healthy. |

These tests indicate that the infective principle of the mosaic disease of tobacco is highly resistant to extremely low temperatures.

## DISAPPEARANCE OF PEROXIDASE IN MOSAIC VIRUS WITHOUT LOSS OF INFECTIOUS PROPERTIES

It has been observed in several instances that unpreserved solutions of virus, as well as dried and ground mosaic material, may lose their peroxidase activities and still retain infectious properties. This happened with dried and ground mosaic leaves bottled in December, 1912. This material showed fair peroxidase reactions on January 28, 1915, but no reactions for peroxidase in October, 1915. At this time the virus still retained the power to produce infection.

In another instance a bottle of unpreserved virus which was extracted on April 27, 1914, failed to give peroxidase reactions on December 3, 1914; yet at this time was highly infectious, producing the disease in 9 plants out of 10 inoculated. This virus was also highly infectious when tested on May 15, 1915, producing the mosaic disease in 8 out of 10 plants. Although the virus was not tested for peroxidase at the time it was extracted, the fresh virus would probably have shown peroxidase

reactions. In the writer's experience, freshly extracted sap from healthy plants as well as from plants affected with the mosaic disease has never failed to give more or less intense peroxidase reactions.

Various experiments have shown that talc-treated virus slowly loses its peroxidase activities, although still retaining its infectious properties, as shown in the following test: On November 19, 1915, 50 c. c. of virus $X^{20}$, extracted on November 1, 1915, and filtered through paper, were mixed to a thick paste with 72 gm. of powdered talc, U. S. P. This material was tested for peroxidase reaction and infectivity from time to time with the following results:

| | | |
|---|---|---|
| Strong peroxidase reaction | Nov. 19, 1915. | |
| Very weak peroxidase reaction | Nov. 30, 1915. | |
| Very faint peroxidase reaction | Dec. 20, 1915 | 10 plants mosaic. |
| No peroxidase reaction | Jan. 18, 1916 | 4 plants mosaic. |

Similar results have been noted when the virus, and also green mosaic material, have been buried in the soil. The virus, and likewise the green material, may entirely lose their peroxidase activities on decaying, although still retaining the power to produce infection.

## INFECTIOUS PROPERTIES LOST AND PEROXIDASE ACTIVITIES RETAINED

While the infective principle of the mosaic disease appears to be very resistant, the infectivity of a virus solution may be lost under some conditions, although the peroxidase is not appreciably changed. This was noted as a result of evaporating a quantity of virus to dryness. A solution of 350 c. c. of virus which had been extracted some time previously and allowed to undergo free fermentation was evaporated at room temperature from September 18 to October 21, 1915. On October 21 the solution had been reduced to 20 c. c. of a thick, heavy, putrid-smelling black sirup. Although this solution gave much more intense reactions for catalase and peroxidase than the original solution, showing that these enzyms had been concentrated during the process of evaporation, the infective principle of the virus had been completely destroyed. Inoculation tests showed that the original virus, however, still retained its infectious properties.

Although in this instance the infective principle had been destroyed, many tests have shown that the virus of the mosaic disease is not usually destroyed, even when evaporated to dryness.

Experiments with the feces of hornworms fed upon the leaves of mosaic plants have given rather interesting results. After the worms had been feeding upon the plants for a day or two the feces were collected and macerated with distilled water. In one test the feces of a single worm were used. Out of 10 plants inoculated, one plant only became diseased. Since but one case of the mosaic disease appeared in this test, there is a possibility that this plant developed the disease as a result of accidental infection from other sources.

In another experiment six hornworms which had been feeding upon mosaic plants in the field were transferred to mosaic plants in the laboratory and left for a day or two. The feces were then collected, macerated with tap water, and tested as follows: Ten plants were inoculated with the extracted sap of mosaic leaves upon which the worms were allowed to feed. Nine plants became mosaic. Ten plants were inoculated with a water extract of the feces of the hornworms. All remained healthy. Ten plants were inoculated with tap water (control). All remained healthy.

Although these results indicate that the infective principle of the original material had been destroyed by the digestive process of the worms, the feces gave intense peroxidase reactions.

## INFECTIVE PRINCIPLE OF THE DISEASE NOT A NORMAL CONSTITUENT OF THE SAP OF HEALTHY PLANTS

Woods, from his cutting-back experiments with tobacco and other plants, was led to believe that the mosaic disease of tobacco had its origin within the cells of the plants as a result of abnormal physiological activities. Although Woods ascribed the origin of the disease to peroxidase, he believed that there was no essential difference between the peroxidase of healthy and that of diseased plants and came to the conclusion that this enzym obtained from either source could produce the disease.

In an earlier paper (1) the writer has adduced evidence to show that the disease is not produced by simply cutting back or otherwise subjecting plants to unfavorable conditions. In the present paper it has also been shown that peroxidase bears no essential relation to infection and that by various methods this and other enzyms may be more or less completely removed from the virus without affecting the infective principle of the disease, and vice versa.

Although the sap of healthy plants may be rich in oxidase, peroxidase, and catalase, such sap never produces the mosaic disease in healthy plants. Although the peroxidase of diseased plants may be decreased to such an extent by dilution with distilled water that it can not be detected by the guaiac-hydrogen-peroxid test, the solution still remains highly infectious. The results of the experiments in which the virus was diluted with distilled water make this plain (Table XV).

TABLE XV.—*Effect of dilution of mosaic virus with distilled water*

| Degree of dilution. | Peroxidase reaction. | Number of plants inoculated. | Result. |
|---|---|---|---|
| Virus undiluted | Intense | | |
| 1 part virus in 250 parts water | Weak | | |
| 1 part virus in 500 parts water | None | 10 | 8 mosaic. |
| 1 part virus in 1,000 parts water | ...do | 10 | 6 mosaic. |
| Tap water only (control) | | 10 | All healthy. |

On the other hand, by evaporation the enzyms present in the sap of healthy plants may be brought to the highest possible concentration, but such solutions never acquire infectious properties.

That oxidase (producing the blue color with guaiac alone) can not be responsible for the mosaic disease may be shown by heating the solution to 70° C. for several minutes. This temperature destroys the oxidase, according to Loew (15, p. 31), but does not affect the peroxidase or the principle of infection. As a matter of fact, the oxidase of the tobacco sap appears to be an unstable enzym and very soon disappears entirely from untreated solutions on standing.

Although the enzym termed "catalase" by Loew is very often a normal constituent of healthy and mosaic plants, it can be shown that the presence of this enzym has nothing to do with infection. As shown by Loew (16, p. 19), catalase is destroyed by heating the solution for a minute or two at 80° C. Such solutions, although no longer showing reactions for catalase, may yet retain their infectious properties.

Although it is known that other enzyms than oxidase, peroxidase, and catalase occur normally in the sap of healthy tobacco plants (18), such enzyms can not be considered in a causal relationship to the mosaic disease if it has been established that this disease is not of so-called physiological or spontaneous origin—that is, it can not occur in the absence of infection. Furthermore, the writer sees no reason to believe that any specific enzym occurs in a mosaic tobacco plant which would not be found in healthy plants.

Although it has been shown by various workers that the enzymic relations and reactions in plants become disturbed as a result of disease and unfavorable conditions of growth, there is no reason to believe that these disturbances, when associated with the mosaic disease of tobacco, hold a causal relation to the disease. It is now well known that various factors, aside from pathological conditions caused by an unknown infective principle, may change the quantitative relations of enzyms in plants, as Bunzel (5) has shown in studying the curly-top of sugar beets. It yet remains to be shown that an increase in the amount or activity of enzyms in diseased plants is anything more than a symptom or an indication of disturbed metabolism as a result of the disease.

In the writer's experience all evidence at hand indicates that the mosaic disease of tobacco is dependent upon a specific pathogenic agent which must be introduced into healthy plants from without before the disease can arise. That this pathogenic entity is highly infectious and is in some manner reproduced within the plant are established facts. If these facts are interpreted according to those fundamental principles upon which all our scientific conceptions in pathology and biology are based, that infectious diseases are associated with parasitism and that self-reproduction is a characteristic of living things alone, it must be admitted that the pathogenic agents responsible for the mosaic disease of tobacco must be parasites. If from the facts stated above it is held that nonliving chemical substances such as enzyms or toxins engender the disease, our fundamental biological conceptions no longer hold true.

SUMMARY

In this paper are given the results of a study of the properties of the virus of the mosaic disease of tobacco, and evidence is adduced to show that the infective principle can not be identified with peroxidase. Briefly, the facts obtained may be stated as follows:

(1) The infective principle of the mosaic disease of tobacco is retained by the Livingstone atmometer porous cup used as a filter, and also by powdered talc. Although the filtrates may show intense peroxidase reactions, they no longer produce infection.

(2) The infective principle of the disease is quickly destroyed in alcohol of a strength of 75 to 80 per cent. In this strength precipitation of the peroxidase is complete. By filtering the solution the peroxidase may be collected, freed from alcohol by evaporation, and redissolved with water. This solution gives intense peroxidase reactions, but no longer produces infection. Alcoholic solutions of virus of 45 and 50 per cent strengths did not destroy the infective principle of the disease within the same period. In these solutions the pathogenic agents are not destroyed and appear to be carried down with the precipitate, leaving the supernatant solution without infectious properties, although giving strong peroxidase reactions.

(3) By the addition of different quantities of hydrogen peroxid to the virus, it is possible to find a concentration of sufficient strength to destroy the peroxidase, but leaving little or no free peroxid in the solution. Such solutions no longer show peroxidase reactions, but retain their infectious properties for a long time. A considerable excess of hydrogen peroxid destroys the infective principle itself. The quantity of hydrogen peroxid required to destroy the peroxidase without leaving any considerable excess in the solution depends upon the nature of the virus, the amount of active catalase present, etc.

(4) The virus was treated with formaldehyde for 31 days in the following concentrations: One part formaldehyde in 100, 200, 400, 600, 800, 1,000, 1,200, and 1,500 parts of virus solution. The solutions containing 1 part formaldehyde in 800, 1,000, 1,200, and 1,500 parts of solution gave infection. Stronger concentrations were no longer infectious, although giving intense reactions for peroxidase.

(5) Ether, chloroform, carbon tetrachlorid, toluene, and acetone failed to extract either the infective principle or the peroxidase from dried mosaic material. These solvents also failed to destroy the infectious principle in this material. Ethyl and methyl alcohol completely destroyed the infective principle in the leaf material itself, as well as in the extract. No evidence of peroxidase was obtained in the alcohol extracts. Glycerin does not destroy the infective principle of the disease. Water extracts of dried material not only show peroxidase reactions, but also contain the infective principle of the disease.

(6) A precipitate of aluminum hydroxid, formed by adding aluminum sulphate to alkaline solutions of virus, appeared to carry down the infec-

tive principle of the disease, leaving the clear, supernatant solution without infectious properties, although showing good peroxidase reactions. Similar treatment with nickel sulphate was not so satisfactory, as it gave evidence of being more toxic to the infective principle than the aluminum salt.

(7) The virus is quickly killed at temperatures near the boiling point of water. In some instances heating the virus for five minutes at 80° C. was sufficient to destroy its infectivity. In other tests, with a different virus solution, heating for five minutes at 90° did not entirely destroy its infectivity. In dried and ground mosaic material, rendered water-free by drying over sulphuric acid in a desiccator, the infective principle resisted much higher temperatures than it did in solutions. If virus solutions are heated, the thermal death point of the infective principle is lower than that of the peroxidase; or at least it is more quickly destroyed than the peroxidase.

(8) The virus is highly resistant to low temperatures. When frozen to a temperature of −180° C. with liquid air, its infectious properties were not weakened.

(9) Unpreserved solutions of virus have sometimes lost their peroxidase activities without losing their infectious properties. Dried and ground mosaic material has also lost its peroxidase activities and still remained highly infectious. Talc-treated material, while retaining its infectious properties, has lost its peroxidase activities.

(10) Solutions of virus sometimes lose their infectious properties and continue to show intense peroxidase reactions, as when allowed to evaporate spontaneously in one instance. The feces of worms fed upon mosaic plants have, in some instances, failed to produce infection, although such material continued to give intense peroxidase reactions.

(11) The writer's experiments show that peroxidase or catalase in the sap of mosaic plants can not be responsible for the mosaic disease. The same enzyms are normally present in healthy plants, but the sap of such plants is without infectious properties. By evaporation the enzyms present in healthy sap may be brought to a high concentration, and such solutions never acquire infectious properties. By dilution, on the other hand, the peroxidase content of mosaic sap may be diminished to such an extent that peroxidase reactions are no longer discernible; yet such solutions may remain highly infectious.

Since it has been shown that the mosaic disease of tobacco does not occur in the absence of infection, neither enzyms nor other normal constituents in the sap of healthy plants can be considered responsible for the disease. A specific, particulate substance not a normal constituent of healthy plants is the cause of the disease. Since this pathogenic agent is highly infectious and is capable of increasing indefinitely within susceptible plants, there is every reason to believe that it is an ultramicroscopic parasite of some kind.

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## PLATE XCI

Livingstone atmometer porus cup as used for filtration. The virus of the mosaic disease of tobacco always lost its infectious properties in passing through this filter.

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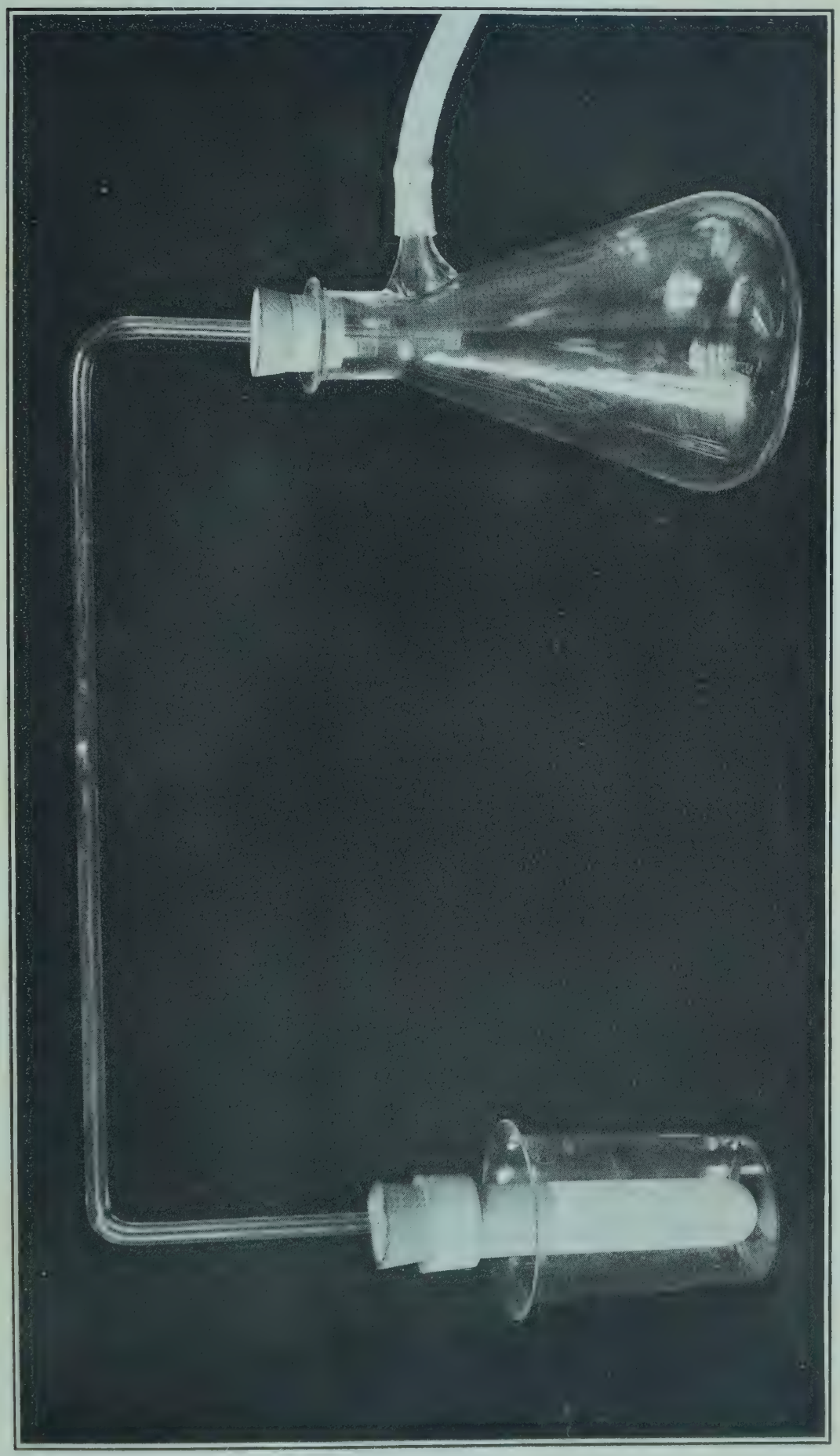

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# LIFE CYCLES OF THE BACTERIA

[PRELIMINARY COMMUNICATION]

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## INTRODUCTION

Two years ago the senior author, together with J. Hanzawa (14),[2] published the results of some Azotobacter studies, showing for the first time that the large Azotobacter cells are a special type of growth of a spore-forming bacillus. We said (14, p. 2):

Es steht jetzt fest, dass in der Tat die grossen, sporenfreien Azotobacter-Zellen Wuchsformen eines schlanken, Endosporen bildenden Bacillus sind. Bacillus Azotobacter ist demnach die korrekte Bezeichnung für diese Art.[3]

As we had to discontinue our investigations at that time, we pointed out (14, p. 6) that further research in this direction would be very desirable:

Sicherlich würden weitere Forschungen in dieser Richtung noch manchen für den Systematiker wie für den Physiologen gleich wichtigen Einblick erschliessen.

In the meantime some new papers on Azotobacter have been published. But they merely confirmed once more certain facts concerning the normal growth and the rather general occurrence of Azotobacter in soils. Only one author, Mulvania (15), reports the presence of heat-resisting spores. In the other cases no spores were observed. However, they undoubtedly would have been found by a more thorough search. One of these authors readily admitted this fact in a letter, saying that his statement had been made "on the basis of the ordinary examination always made by soil bacteriologists."

[1] The photomicrographs, as well as the final drawing of the text figure accompanying this paper, have been made by Mr. F. L. Goll, of the Bureau of Plant Industry. Grateful acknowledgment is due him for his very careful work.

[2] Reference is made by number to "Literature cited," p. 701–702.

[3] In accordance with the usage of K. B. Lehmann and most of the other European bacteriologists, we apply the name "Bacillus" to the spore-forming rods. As *Bacillus azotobacter*, like most, probably all, spore-forming rods, has, at least temporarily, peritrichous flagella, its name would also be valid if Migula's system should be preferred. However, we fully agree with Lehmann, that this system is especially unsatisfactory.

As our earlier observations indicated that an extended study would lead to still more interesting results, we have resumed our work. A comparative study of 24 Azotobacter cultures and 18 strains of other bacteria now revealed the fact that those wide morphological differences first observed with Azotobacter are by no means restricted to this one group of bacteria. Similar variations occurred with all cultures tested, and under suitable conditions they will occur with all bacteria generally. The importance of these wide morphological variations, however, is materially increased by the fact that they are connected with no less considerable variations in the physiological qualities of those organisms. Therefore, not only for diagnostic and systematic purposes are these facts of fundamental importance but also for all other lines of research in agricultural and medical bacteriology.

The quite unexpected character of the results obtained seems to justify a preliminary discussion of the facts and problems involved. Of course, at the present time it is neither our intention to furnish all those numerous details which are necessary to obtain a full knowledge of these heretofore practically unknown facts, nor do we want to collect all the widely scattered observations from a voluminous literature which will not only give some interesting support to our new viewpoint but which also, in their turn, will sometimes find their full explanation there. At present we merely wish to inform agricultural and also medical bacteriologists about these newly discovered facts and to ask for their cooperation.

It is beyond question that progress in bacteriology has been severely checked by the widespread inclination to consider as not worth studying or as some uninteresting "involution form" all that sort of bacterial growth which does not fit exactly into the conventional conception of a very simple and constant character of the species. Even modern standard works assert, for instance, that the branched type of *Bacillus radicicola* represents an "involution form" not capable of further propagation. However, nitrogen fixation takes place only when these branched forms develop, which unmistakably proves their full virility; and there is no lack of exact results which show conclusively that suitable conditions always allow a new development from these branched forms.

Undoubtedly a somewhat more scientific study of such "abnormal" forms would long ago have revealed the fact that the life cycles of the bacteria are no less complicated than those of many other micro-organisms. Indeed, numerous items in the bacteriological literature, for instance, show that the formation of gonidia and the budding of bacteria have been observed quite frequently. Yet again the authoritative statement that bacteria multiply exclusively by fission apparently has been sufficient to prevent thorough research in this direction, and the credulous adherence to "standard methods" unfortunately explains only too well why the turning point in the life cycles of the bacteria has been com-

pletely overlooked. In fact, this slime or granulated dirt has been merely an annoying occurrence on the slides of thousands of bacteriologists. Acetic acid and many other remedies have been recommended to insure clean preparates. Of course, beef broth and some other substrates usually give really dirty smears which need some cleaning, but we have been much too radical in this direction. Under certain conditions all bacteria pass over into a "symplastic" stage, appearing under the microscope as either an unstainable or a readily stainable mass without any visible organization, which, if not discarded as dead, later gives birth to new regenerated forms frequently of very characteristic and unusual appearance.

As practically all our new knowledge of the life cycles of the bacteria has been derived from a renewed study of *B. azotobacter*, the behavior of this organism will be described first.

Before we enter into this subject, however, we beg to point out that by discussing the life cycles of the bacteria we do not intend to revive any of those unclear theories concerning bacterial polymorphism or pleomorphism. The development of the bacteria is characterized not by the *irregular* occurrence of more or less *abnormal* forms but by the *regular* occurrence of many different forms and stages of growth connected with each other by *constant relations*.

Unquestionably many so-called species frequently described in the most superficial manner will have to be canceled, because they merely represent fragments of the life cycles of other bacteria. "Good" species, on the other hand, will not only keep their position, but they will receive a much more complete and sharper definition than they now have. Moreover, the discovery of the symplastic stage opens the way to answer by exact experiments the question concerning species or varieties.

## THE LIFE CYCLE OF BACILLUS AZOTOBACTER

In text figure 1 is given a schematic sketch of the development of *B. azotobacter* according to our present knowledge. The letters A to M indicate the different types of growths which are separated from each other by broken lines. The single- and double-pointed arrows show the connections between the different forms as they have actually been observed. Each of the four circles contain in each case all those forms which have heretofore been considered by careful investigators as representing sufficient basis for establishing a species. Observers of the more usual, less painstaking class, however, have been only too much inclined to form new species even inside these subcycles. For example, the different types of spore-free and spore-bearing rods, all included in our type F, could easily have induced authors like Migula and Matzushita to create half a dozen "species" of that sort; perhaps this really happened.

With the exception of D and H, all these types have been observed and described in earlier publications on Azotobacter and closely related

spore-forming bacilli (*B. malabarensis*, *B. danicus*, and *B. oxalaticus*). However, figure 1 shows clearly how just this type D, which has hereto-

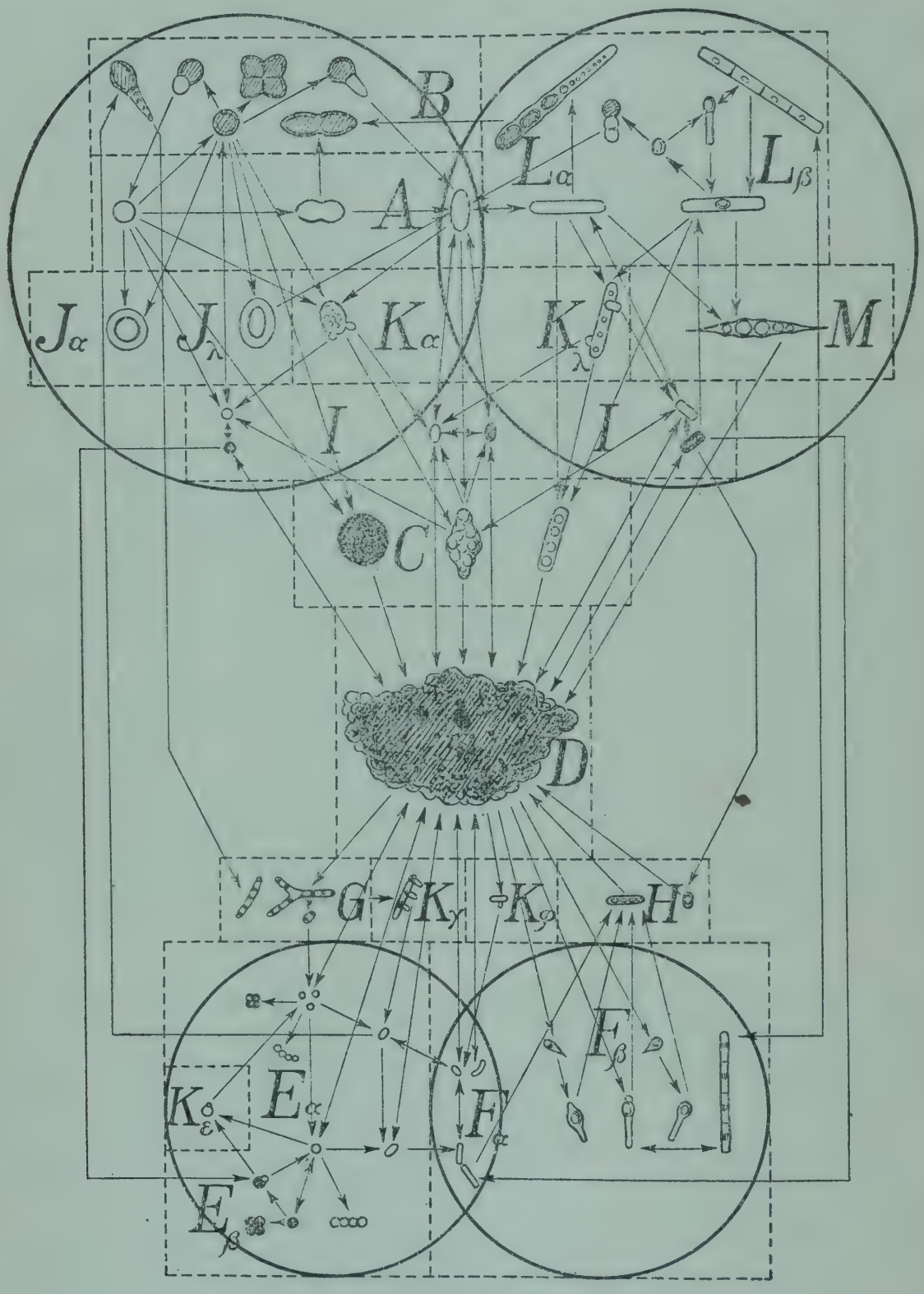


FIG. 1.—Life cycle of *Bacillus azotobacter*. The broken straight lines divide the different types of growth indicated by the letters A to M. The Greek letters α to λ refer to subdivisions. The single- and double-pointed arrows indicate the development of one form from another. The four circles confine, in every case, all those forms which represent together a rather constant mode of life and which have been usually considered as bases for establishing separate species.

fore so successfully dodged all bacteriologists, represents practically the key to a correct understanding of the whole problem. There are some

direct outside connections between the larger and the smaller forms, as indicated by the six arrows: I–E, E–B, B–G, and I–F, F–L, I–H; but they are rather rare exceptions. The symplastic stage D, on the other hand, can be observed in all cultures. Even if a strain shows little or no inclination to change from one subcycle into another, it regularly passes through type D. By frequent successive transfers (made each morning and evening), we tried to prevent this "breakdown." For some days we were successful, but then this disintegration again took place. After another couple of days the tendency to produce "normal" forms was once more very pronounced, which in its turn was again supplanted by the formation of type D. We have followed this rythmic alternation for some weeks with *B. azotobacter* as well as with *B. subtilis*.

It goes without saying that the arrangement and naming of the different types is merely a matter of convenience. Several of them perhaps could be split up into two or more. However, we found them so suitable in their present form for our work that we do not see any necessity for making an alteration.

Type A represents the normal, well-known, large Azotobacter cells of globular or oval form (usually 2 to 3$\mu$ broad and 3 to 5$\mu$ long). By further stretching they pass over into type L (fig. 1 and 2 of Pl. A).

Type B indicates the thick-walled, rather resistant "arthrospores," regularly formed from type A.[1] When they germinate, either globular or oval forms are liberated. Some cells of type B, however, are produced occasionally by D, or still less frequently by a direct enlargement of small cells of type E or F, which will be discussed later. In the latter case the germ developing from B shows the character of type G. Probably no B formed by A germinates in this manner. This fact, then, would distinguish these morphologically identical forms.

Type C comprises all large forms in the stage of granular decomposition heretofore generally considered to be dying "involution" forms. If the granules are very small, they are nearly always easily stained. The larger ones, on the other hand, usually remain entirely unstained when treated with aqueous solutions of anilin dyes. Owing to the degenerated condition of the cell wall, the form of the cells frequently becomes quite irregular, and the granular content may become partially or entirely free (fig. 7 to 10 of Pl. B). Undoubtedly these granules are of different nature. Some may be fat, glycogen, or other metabolic products. Most of them however, are living entities, as is clearly shown by their further behavior, if not by their motility. Sometimes these granules develop to full-sized cells before being liberated. (See type J.) In this case they behave exactly like the "gonidia" in iron bacteria, as described by Cohn (4). However, in most cases they either leave the cell entirely before they

[1] We think it best to reserve the name "arthrospore" exclusively for those cases where the whole cell acquires the character of a spore. If only parts of the cell (either at its end or side) show such transformation, we call them "regenerative bodies" or "exospores," according to their special character.

start growing, or they grow out of it, piercing the cell wall. With all small bacteria we have observed only the two latter types of growth. Almquist (1), who made some similar observations, called these granules "conidia." In our opinion the term "gonidia"—that is, seed—is preferable, as these granules in every case act as organs of propagation and multiplication, whatever may be their special mode of growth.

Type D is in most cases the dissolution product either of the large forms (types C and M) or of the small cells (types E, F, and H), but it can also be formed by typical spores of type L, by regenerative bodies (I) and by gonidia. Its inclination to take the stain varies widely. If the cell walls participate considerably in its formation, it is readily and deeply stained. The same holds true when the gonidia are small and easily stainable. The large unstainable gonidia, on the other hand, which are frequently produced in type C, as well as in type H (see below), naturally give a rather pale or entirely unstained D. The structure, too, varies accordingly. Small cells, or small gonidia, cause a finely granulated, somewhat "hairy" structure; especially in the case of small slender rods like *B. fluorescens*, *B. radiobacter*, etc., the term "woolly" perhaps would be applicable. Large gonidia, on the other hand, as well as spores, clearly melt together when entering this stage of growth. Figures 7 to 12 (Pl. B), 18 (Pl. C), and 19 (Pl. D) illustrate the different possibilities. Like type C, type D has been considered by some investigators—for example, by K. B. Lehmann—as an occurrence indicating the death of the bacteria.[1] Usually, however, it has been passed as some uninteresting "slime" or "dirt." As it is made up by a thorough mixing or melting of a frequently large number of cells, spores, or gonidia, the term *symplasm* or *symplastic stage* seems to be a correct and convenient name for this stage.

Some time after the symplasm has been formed, very small granules, *regenerative units* (0.2 to 0.3$\mu$), become visible. If the symplasm does not take the stain, the appearance of these organized well-stained forms inside the amorphous pale mass is very surprising (fig. 12 of Pl. B). Such a preparate indeed first turned our attention into this new direction. The regenerative units increase in size until they show the form of type E, F, I, or even A or B (fig. 13 and 14 of Pl. C). All these cells are easily stained, their cell walls being usually comparatively thick. At last, practically all the symplasm is reorganized, leaving sometimes only very few pale small "flakes."

Type E represents a miniature counterpart of types A and B. The size of the cells varies between 0.3 and 1$\mu$. Only with the latter forms are the thin and the thick walled cells clearly discernible. In some cases at least, we were able to observe germinating arthrospores of this type. If necessary, both subtypes may be indicated conveniently by appending to the E a Greek letter $\alpha$ or $\beta$, respectively.

[1] The absurd name "zoogloea," which means "animal slime," has been repeatedly applied to this product of bacterial "autolysis," and the fact that the *walls* of the cells are dissolved has been considered as indicating the death of the *content* of the cells.

Type F comprises all small rodlike cells of different form, straight or curved, about 0.3 to 0.5$\mu$ broad, 0.75 to 1.5$\mu$, or more, long. When not forming spores, they may be labeled F$\alpha$; otherwise F$\beta$. Cells of the F$\alpha$ type occasionally look very much like *B. radiobacter* and related species. In cases where great difficulties were encountered in getting a pure culture of *B. azotobacter*, this type of growth probably has repeatedly displayed an unwelcome activity. When developing from the symplastic stage, type F$\beta$ shows different and somewhat unusual-looking intermediate forms (fig. 15 of Pl. C). For making a spore-bearing rod of the "Plectridium" type, a body splits off from the symplasm, showing a comparatively large "head" and a very small pointed "tail." When the tendency prevails, however, to form a "clostridium", the well-stained regenerative unit is located inside a pale sheath with pointed ends and in growing stretches until the albuminous substance is equally distributed inside the cell wall. Later, a part of the protoplasm once more concentrates, developing the spore. Spore-free, as well as spore-bearing, thin threads can directly, without passing through type D, change into the large type L. (See below.) On the other hand, they can also originate directly from this type (fig. 20 of Pl. D). In the latter case they sometimes resemble type G, from which they differ, however, by their spore formation and their genesis.

Type G shows, when treated with aqueous fuchsin, unevenly stained, frequently branched threads looking very much like Actinomyces. It is, however, as indicated in text figure 1 and shown in figure 16 (Pl. C), merely an intermediate step between types D and E which may be dispensed with. The small cells of type E are kept together by unstainable slime. Boiling water dissolves this slime within two minutes. A preparate treated accordingly with boiling aqueous fuchsin shows merely type E or some threads just dissolving (fig. 17 of Pl. C).

Type H acts as the counterpart of type C. There the larger, here the smaller cells are undergoing a granular decomposition leading to type D. However, rods, as well as spores, show a very unusual appearance in this case. They become entirely unstainable by aqueous dyes, but remain clearly visible even with a wide-open condenser, owing to the very bright luster of their granular content (fig. 18 of Pl. C).

Type I represents the globular, oval, or rodlike "regenerative" bodies which have been studied by Prazmowski (18). Here, again, an added $\alpha$ or $\beta$, respectively, may indicate their more or less resistant character (thin or thick cell wall). I$\alpha$ usually originates from types A and B (fig. 5 of Pl. A) or from type C (fig. 7 of Pl. B). I$\beta$, on the other hand, in most cases is an offspring from the symplastic stage D (fig. 13 of Pl. C). Irregularly shaped type I, which is quite frequent with the other bacteria, has been observed only occasionally in cultures of Azotobacter (fig. 16 of Pl. C). The regenerative bodies either produce, by germinating or by stretching, cells of type A, B, or L or they convert themselves entirely into

regular spores. This possibility will be discussed at once. But, as mentioned before, regenerative bodies may also produce forms belonging to types E and F, making two of the "outside" connections between the upper and the lower circles as given in text figure 1.

Type J characterizes another rather rare occurrence also studied by Prazmowski. Forms belonging to type A, B, or occasionally L, increase in size and inside themselves develop one or more new full-sized cells of type A or B (fig. 6 of Pl. A and fig. 21 of Pl. D). These new cells are the result of the growth of the gonidia.

Type K comprises all those cells of type A, B, E, F, G, I, or L which produce one or more well-stained, round, oval, or rodlike buds, which, in the case of the large forms, occasionally cause a close resemblance to some budding yeast cell. These "buds" are gonidia developing into regenerative bodies, seldom directly into full-sized forms. An added $\alpha$, $\beta$, $\epsilon$, $\varphi$, $\gamma$, $\iota$, or $\lambda$ indicates the relation to type A, B, E, F, G, I, or L, respectively (fig. 3 to 6 of Pl. A; fig. 14, 16, 17 of Pl. C; fig. 20, 21, 24 of Pl. D).

Type L is made up of the large spore-free and spore-forming rods L$\alpha$ and L$\beta$, as well as of free spores and long threads. Germinating spores of this type produce either long rods or rather short ovals resembling type A. The big spore-free rods and threads resulting from type A (fig. 1, 2 of Pl. A) seem to be unable to develop directly the faculty to form endospores. At least, we have never observed such a change, and this also would be in accordance with the fact that a direct transformation of a spore-free into a spore-forming bacterium has never been observed. As mentioned above, type F$\beta$, too, does not develop from type F$\alpha$, but directly from the symplasm. As is also noted above, these small spore-forming rods occasionally convert themselves into large spore-forming bacilli. Usually, however, the regenerative bodies formed by type D seem to be the normal predecessors. Under conditions, which will have to be studied more closely, these round cells acquire the tendency to produce endospores, which, in their turn, go back into the symplastic stage. This second symplasm then produces another set of regenerative bodies which stretch out to large granulated rods and threads. They later form the normal endospores.

Type M represents another rather unusual form. It originates from type L and passes over into type D (fig. 10 of Pl. B and fig. 19 of Pl. D).

It is hardly necessary to point out that sometimes our separation of the forms observed into different types becomes more or less arbitrary. For example, there are no absolutely sharp lines separating types A and B or the regenerative bodies I from the full-grown cells. Thick-walled cells of types E and F, when produced in the symplasm, might just as well be considered as "regenerative bodies." A germinating cell of type E$\beta$ and a budding form of the type K$\epsilon$ resemble each other very

closely. However, similar difficulties occur in all such cases, where we want to reach some clear ideas concerning the multitude of the varying forms which we find in nature; and, as we now know that the life cycle of the same bacterium presents so many more different aspects than we ever expected before, we simply are compelled to take our refuge in some kind of classification, however crude it may be.

In Table I we give a summary of the different types of growth observed thus far in our preliminary studies with 24 representatives of the group of *B. azotobacter*. The laboratory numbers of the different cultures are to be connected in the following manner with the different types of Azotobacter, according to the denominations generally used:

No. 1, 2, 10, 11, 12, 14, 17 to 20, *Azotobacter chroococcum*, old stock cultures.

No. 21 to 25, *Azotobacter chroococcum*, new cultures isolated from different soils.

No. 3 to 6, 13 and 15, *Azotobacter Beijerinkii.*

No. 7 and 16, *Azotobacter vinelandii.*

No. 9, *Azotobacter vitreum.*[1]

TABLE I.—*Types of growth observed with 24 cultures of Azotobacter*

[The laboratory numbers of the cultures are given at the head of the columns]

| Types of growth. | *A. chroococcum*, old stock cultures. | | *A. Beijerinkii.* | | | | *A. vinelandii.* | *A. vitreum.* | *A. chroococcum*, old stock cultures. | | | *A. Beijerinkii.* | *A. chroococcum*, old stock cultures. | *A. Beijerinkii.* | *A. vinelandii.* | *A. chroococcum*, old stock cultures. | | | | *A. chroococcum*, new cultures isolated from different soils. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
| A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| B | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C | + | + | + | + | + | ... | + | + | + | + | + | + | + | ... | ... | ... | + | ... | ... | + | + | + | + | + |
| D | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| E | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| F | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| G | ... | + | ... | + | ... | + | + | ... | + | + | + | + | + | + | ... | + | + | + | ... | ... | + | ... | ... | + |
| H | + | ... | ... | ... | ... | ... | + | + | ... | ... | + | ... | + | ... | ... | + | + | ... | ... | + | + | + | + | + |
| I | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| J | ... | ... | ... | ... | ... | ... | ... | ... | ... | + | + | ... | + | ... | ... | ... | + | ... | ... | ... | + | + | + | ... |
| K | + | + | + | ... | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| M | ... | ... | + | ... | ... | ... | ... | + | ... | + | + | ... | + | ... | ... | ... | + | ... | ... | ... | + | + | ... | ... |

[1] Another strain of *A. vitreum* isolated in 1904 in Leipzig bore the No. 8 (now missing) in our collection. It died shortly before these investigations were started. One of the photographs (fig. 6 of Pl. A) was made by using an old preparate of this culture. We hope to be able to replace it later by a new subculture of the original strain left in the laboratory of the senior author in Leipzig.

More complete studies, of course, will fill all places now vacant in the table. The rather irregularly observed types G, H, J, and M are of no fundamental importance in the life cycle of *B. azotobacter*, and our interest has not been especially centered upon them. The large forms (types A, B, and L), as well as the small ones (types E and F), have been found in all cases. The same holds true concerning types D and I. This means that the full life cycle typical for *B. azotobacter* has been observed with every strain. That budding forms (type K) were also noticed in 23 out of 24 cases is of minor interest, because this is another type which represents no essential link in the cycle of bacterial life. Merely its unusual and heretofore nearly unknown appearance naturally attracted our attention.

The growth of the gonidia which causes this "budding" occasionally leads to three other kinds of development which deserve some short discussion. If the "buds" stretch out considerably, they cause the formation of branched bacteria, which can be found in the Azotobacter group, as well as in all other groups of bacteria. If, however, several gonidia, inclosed in the same cell or in its liberated granular content, start growing simultaneously in different directions, starlike forms result, which are frequently found in cultures of rodlike bacteria. *Bact. radiobacter* probably is the best known example of this special type of growth. But we have already reserved for a later publication good pictures of starlike outgrowths of slender rods from the typical large globular Azotobacter forms. An interesting crownlike form, representing the medium stage between simple budding and the formation of stars, is shown in figure 16 (Pl. C) directly above the upper symplasm. The third kind of gonidial development is another heretofore unknown type of growth. The budding gonidia sometimes develop into forms which clearly show the morphological and physiological character of typical endospores. Since they are produced, however, outside of the cell, they may be called *exospores*. In figure 20 (Pl. D) different stages of their development are reproduced. Their occurrence is not quite so surprising as it may seem to be at first, when considered in the light of the two following facts. Those "granules" which precede the formation of the normal endospores inside the bacterial cells are actually nothing else than small gonidia. When budding, the gonidia frequently develop into thick-walled regenerative bodies, which not only germinate in the same manner as endospores but may also acquire quite a considerable resistance against heat. As mentioned above, regenerative bodies growing out of the symplasm sometimes convert themselves practically entirely into spores. Budding exospores therefore are merely a special application of a general rule; they are regenerative bodies with the character of spores.

Normal heat-resistant endospores showing polar germination have been found in 13 of our Azotobacter cultures—that is, in more than 50

per cent of all cases. During our first investigations upon this subject (14) only 4 of 11 strains (No. 2, 4, 5, and 6) possessed this faculty, which they had acquired between 1908 and 1912. Culture 7 gave some bodies looking like endospores in 1914, but they were not resistant to heat. This faculty has now been fully developed. Cultures 15, 17, and 19, also typical spore-free cells at the time of their isolation some years ago, showed their inclination to form endospores when first tested in this direction in 1915. Cultures 23 to 25 developed this special character during the time we were experimenting with them. To fix exactly the conditions for transforming a spore-free into a spore-bearing strain will be one of the tasks in our later, detailed investigations. As already indicated, a close study of the symplasm and of the regenerative bodies derived from it will solve this problem as well as many others concerning the multitude of forms inclosed in the life cycles of the bacteria.

Unquestionably in all such experiments the inner condition of the cells is of no little importance. That at least in some directions this factor can eventually outmatch the influence of the outer conditions to a considerable extent is clearly shown by the interesting behavior of 22 of our Azotobacter strains when they were inoculated into soil extract containing 1 per cent of mannite and 0.05 per cent of monobasic potassium phosphate ($KH_2PO_4$), after having grown previously in moist sterilized soil and mannite. Fifteen of these cultures grew in the large types A and L and seven in the small types E and F when the experiment started. Two weeks later, without a single exception, all the fifteen strains changed from types A, B, and L to D, E, and F. Vice versa, the seven others produced types D and I, these developing into types A, B, and L. This result corroborates once more our statement that every strain of Azotobacter or of any other bacillus will pass through all phases of its cycle of life persistently if the conditions are suitable. Undoubtedly and in full accordance with the behavior of higher organisms, some strains are especially inclined to grow mostly in one or the other subcycle. However, the formation of the symplasm and its "plasticity" enables us, if we make use of these interesting possibilities, to induce and accelerate changes in the general development of a bacillus to which the special strain perhaps may be only very little inclined at that time. For example, our five newly isolated Azotobacter cultures 21 to 25 had, of course, the pronounced tendency to grow in their typical large globular or oval form. Only in about 1-month-old mannite solutions were the long rodlike forms more numerous, mixed with forms belonging to types A, B, D, E, F, and I. Transfers on mannite agar, after one day, gave in four cases an abundant and practically pure growth of the large rods, showing a tendency to form endospores. Only one culture (No. 24) failed, because in this case we had no such old solution at hand and had started, therefore, from a 3-day-old culture, which exclusively produced round and oval regenerative bodies on mannite agar.

Mannite soil extract, which has been used with very satisfactory results by the senior author (13) for more than 10 years for the study of nitrogen-fixing and other soil organisms, unquestionably has the disadvantage of a varying and partially unknown chemical composition. However, beef broth and similar substrates are liable to the same objection and yet are generally used in bacteriological laboratories. Nevertheless it would be preferable to have media at our disposition of the same favorable qualities but of well-defined chemical composition. Practically all of the many artificial substrates recommended in the bacteriological literature are much too concentrated, especially for soil organisms. For many of our experiments we used the following mineral solution to good advantage:

| | |
|---|---|
| Monopotassium phosphate neutralized to phenolphthalein by sodium hydroxid | 0.02 per cent |
| Magnesium sulphate | .02 per cent |
| Sodium chlorid | .02 per cent |
| Calcium sulphate | .01 per cent |
| Ferric chlorid, 1 per cent solution | 2 drops per 100 c. c. |

As carbonaceous material for *B. azotobacter*, 1 per cent mannite was used. The further addition of 0.02 per cent of potassium nitrate or peptone proved to be beneficial though not necessary. All these solutions are entirely clear and therefore especially suitable for microscopical studies. If a strip of filter paper sufficiently long to reach about 1 inch out of the solution is placed into the test tube before sterilizing, a luxuriant growth of the large forms belonging to types A and B quickly spreads on the part above the liquid.[1] In old solutions the symplasm frequently develops to such a degree that it becomes clearly visible to the naked eye as white flakes or slimy threads. Figure 14 (Pl. C) is a reproduction of the end of such an enormous accumulation of living material.

The last three pictures of our Azotobacter series (fig. 22 to 24 of Pl. D) illustrate one of the comparatively rare direct connections between the small and the large forms. We have here before us the exact counterpart of the alteration shown in figure 20 (Pl. D). Certainly this direct growing up of the small organisms to forms belonging to type B, the forthcoming of threads of type G in the germinating process, and the unusual appearance of their budding, by which the small forms are regenerated, deserve our full attention. However, this kind of development seems to be a rather rare exception to the rule. These forms also are much inclined to turn into the symplastic stage. A photographic picture of this occurrence will be published later.

The close study of this side connection, however, led us to another discovery which we had failed to make before, although our other preparates,

[1] The arrangement mentioned above is very helpful for obtaining pure cultures of Azotobacter from the soil. At the same time it allows the motility of an organism to be determined macroscopically. One of our strains crept up 20 cm. in 10 days on long paper strips in large test tubes.

as reproduced in figures 1 to 3 (Pl. A) and 8 (Pl. B), show the same fact much more clearly. In all these preparates many cells are in a conjunct stage, which can not be explained by the assumption that this *conjunction*[1] is only accidental. Most of these illustrations have been made from contact preparates taken directly from 4-day-old colonies. Smears made in accordance with the "standard methods" probably would have destroyed most of these connections. But also in this case it was still more the effect of our theoretical blinders which prevented an earlier seeing and understanding of this fact, which, like the budding of the bacteria and the formation of the symplasm, has not only actually been seen by many bacteriologists but also has been unknowingly reproduced in several illustrations in our daily used textbooks.

So far as we are aware, only one author has spoken of a similar observation. In 1892 Förster (6) found occasionally that *Chromatium Okenii* sometimes entered into some "primitive copulation." Among the drawings accompanying his paper, a sketch made from a photomicrograph seems to us most trustworthy. Its conformity with our Azotobacter illustrations is practically complete. Observations in the hanging-drop clearly showed that there is some interference between the plasmatic substances in the conjunct cells or even some direct mixing of them.

The determination of the actual physiological significance of this conjunction must be left, of course, to a more thorough investigation. At present we merely wish to add and to emphasize that this process is by no means such an exception as might be deduced from Förster's statements and from the silence observed in this direction in our textbooks. The conjunct stage seems to be of no less general importance and occurrence in bacterial life than the formation of the symplasm. Not only normal cells and regenerative bodies but also exospores have been frequently found in conjunction. And if we only succeed in forgetting for a moment our most cherished theories and simply try to look at the facts as they are, we find at once that the formation of the symplasm and the conjunction of the cells are nothing else than two modes of mixing plasmatic substances temporarily inclosed in separate cells and that evidently the continuity and rejuvenescence of the living matter in the bacteria is just as much dependent on this process as in the case of all other organisms.

A thorough study of the relations existing between the conjunct and symplastic stage will be the first object of our further investigations in this line. We hope that experiments with well-defined varieties and species will soon furnish a correct insight. The ease with which the "flakes" of the symplasm can be isolated is, of course, very advantageous for these, as well as for systematic, studies.

[1] We prefer the new term *conjunction* instead of "copulation" or "conjugation," because frequently more than two cells unite and no sexual differentiation so far has been observed.

Before entering a discussion of the life cycles of other bacteria, the serial numbers for the four subcycles of *B. azotobacter* may be given, determined, so far as possible, in accordance with the methods recommended by the Society of American Bacteriologists. The behavior in the presence of the different carbonaceous substances, however, had to be tested in our mineral solution with nitrate to which 0.5 per cent of the different sugars, etc., was added, the highly concentrated peptone solution not being suitable for this organism. That the appearance of the colonies, as well as the other cultural characteristics, differs accordingly, goes without saying; these details will also be given later. The serial numbers resulting from our tests are as follows:

Type A.—221.2322813. Type E.—222.2222524.
Type L.—121.3332033. Type F.—122.4442034.

## THE LIFE CYCLES OF OTHER BACTERIA

The following 18 cultures were selected as representatives of practically all groups of bacteria.

No. 31. *B. subtilis*, isolated from evaporated milk.
No. 32. *B. lactis niger*, Gorini's original culture from Kral's Museum.
No. 33. *Tyrothrix tenuis*, Duclaux' original culture from Kral's Museum.
No. 34. *B. danicus*, isolated from soil.
No. 35. *Bact. pneumoniae*, isolated from soil.
No. 36. *Bact. radiobacter*, isolated from soil.
No. 38. *Bact. denitrificans agile*, Ampola's original culture from Kral's Museum.
No. 39. *Bact. radicicola*, isolated from vetch.
No. 40. *Bact. fluorescens*, isolated from milk.
No. 41. A yellow bacillus (not determined) isolated from soil.
No. 42. *Planosarcina ureae*, Beijerinck's original culture from Kral's Museum.
No. 43. *Sarcina flava*, isolated from milk.
No. 44. *Micrococcus candicans*, isolated from chernozem.
No. 45. *Micrococcus candicans*, isolated from evaporated milk.
No. 46. Salt-water spirillum isolated from Great Salt Lake, Utah.
No. 47. Salt-water spirillum isolated from sea water.
No. 48. *Streptococcus lactis*, kindly furnished by Dr. L. A. Rogers, Bureau of Animal Industry.
No. 49. *Bact. bulgaricum*, kindly furnished by Dr. L. A. Rogers.

Before being tested, these cultures had been grown on the following substrates:

Beef agar: No. 31–36, 38, 40, 41, 43–45.
Beef agar plus 3 per cent of sodium chlorid: No. 46, 47.
Beef agar plus 0.5 per cent of urea: No. 42.
Saccharose agar: No. 39.
Milk: No. 48, 49.

After having been examined as to their purity on agar plates, they were cultivated on the different agars and in suitable solutions. Beef, salt, and urea agars were used as before. In the case of *B. radicicola* (No. 39),

however, the saccharose agar was substituted by mannite agar as prepared for *B. azotobacter*. The milk organisms (No. 48 and 49) were transplanted on yeast-whey agar and into yeast-whey solution, these media being the most suitable for these organisms, according to the earlier observations of the senior author (13). The salt-water spirilla were grown in beef broth with 3 per cent of sodium chlorid. For the other organisms various solutions were prepared by adding to the mineral solution as used for *B. azotobacter* the following ingredients:

0.1 per cent of ammonium citrate plus 0.3 per cent of glycerin for cultures 31, 33 to 36, 38, 40, 41, 44, and 45.
0.04 per cent of peptone plus 0.3 per cent of glycerin for cultures 32, 42, and 43.
0.02 per cent of potassium nitrate plus 1 per cent of mannite for culture 39.
0.04 per cent of peptone plus 0.5 per cent of lactose for cultures 48 and 49.

All cultures were kept at 28° C. with the only exception of those of *Bact. bulgaricum* (No. 49), for which a temperature of 40° to 45° C. is more suitable.

In the light of the results obtained by us with *B. azotobacter* it was not difficult to find out that the life cycles of all these organisms are essentially the same. On all good substrates they all pass quite regularly through most, if not all, of the types of growth first observed with *B. azotobacter*. In Table II the results are summarized. Type N, which we have added here, represents the starlike growth previously mentioned.

TABLE II.—*Types of growth observed with 18 representative bacteria*

[The laboratory numbers of the cultures are given at the head of the columns]

| Types of growth. | *B. subtilis.* | *B. lactis niger.* | *Tyrothrix tenuis.* | *B. danicus.* | *Bact. pneumoniae.* | *Bact. radiobacter.* | *Bact. denitrificans agile.* | *Bact. radicicola.* | *Bact. fluorescens.* | Yellow bacillus. | *Planosarcina ureae.* | *Sarcina flava.* | *Micrococcus candicans* (soil). | *Micrococcus candicans* (milk). | Salt Lake spirillum | Ocean spirillum. | *Streptococcus lactis.* | *Bact. bulgaricum.* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 31 | 32 | 33 | 34 | 35 | 36 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 |
| A (large globules and ovals) | + | ... | ... | + | ... | ... | ... | ... | ... | + | + | ... | ... | ... | ... | ... | ... | ... |
| B (thick-walled forms of type A) | + | + | + | + | ... | ... | ... | ... | ... | + | + | ... | ... | ... | ... | ... | ... | + |
| C (granular decomposition of A, B, L, M) | + | + | + | + | ... | ... | ... | ... | ... | + | + | ... | ... | ... | ... | ... | ... | + |
| D (symplasm) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| E (small globules and ovals) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| F (small rods and threads) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| G (slime threads with cocci) | ... | + | + | + | + | + | + | + | + | + | + | + | ... | ... | ... | + | ... | + |
| H (granular decomposition of F and spores) | + | + | + | + | + | + | + | + | + | + | + | ... | + | ... | ... | ... | ... | + |
| I (regenerative bodies) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| J (normal cells developing inside) | + | ... | + | + | ... | ... | ... | ... | ... | + | ... | ... | ... | ... | ... | ... | ... | ... |
| K (budding gonidia) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L (large rods and threads) | + | + | + | + | ... | ... | ... | ... | ... | + | + | ... | ... | ... | ... | ... | ... | + |
| M (cells with pointed ends) | ... | + | ... | + | ... | + | + | + | + | ... | ... | + | + | + | ... | ... | + | + |
| N (starlike growths) | + | + | ... | ... | + | + | + | + | + | + | ... | ... | + | + | + | ... | ... | + |

The disintegration of the normal cells into the symplastic stage (D), the formation as well as the further development of regenerative bodies

(I), and the occurrence of gonidia budding out of the normal cells (K) have been observed in every case. With all those cultures which regularly produce endospores (No. 31–34, 41, 42), large cells belonging to types A, B, C, and L, were observed; and they were not observed with the same constancy in cultures 35–40, 43–48. Of all these organisms none are known to produce endospores. *Bact. bulgaricum* (No. 49), too, is spore-free; but closely related forms isolated from the stomach have been reported as producing endospores. The formation of cells of type B, C, and L, makes it highly probable that an experimental trial to induce spore formation may soon be successful. However, the same possibility is by no means excluded in the other cases. It may be that small spore-forming forms can be branched off from the other cultures. Indeed, we have already obtained some quite encouraging results in this line. Whether, then, another progression to the large cell types will be possible is entirely an open question.

Referring again to our introductory remarks, we take this opportunity to point out specifically that these perhaps somewhat surprising statements should by no means be considered merely as some absurd polymorphistic hypothesis. The well-known character of *Bact. pneumoniae*, for example, will by itself remain completely unchanged, whatever may be the result of further investigations upon the full life cycle of this organism. If there is a spore-forming type, and perhaps even genetic relations with some large-sized cells, this would in no way interfere with nor impair the well-established facts already collected. Such wide morphological differences must always be connected with no less considerable alterations of the whole physiological character, so that these other types, if they are known, of course, are stored away as entirely different "species" in various remote places in the so-called "system" of bacteria. This conclusion can be drawn with absolute certainty from our observations on *B. azotobacter* as well as from Henri's experiments (10) with *B. anthracis*. If only those changed forms, frequently seen in all bacteriological laboratories, had not been persistently discarded as uninteresting "involution forms" or as "contaminations," the whole situation would undoubtedly be much clearer and much more satisfactory. At present it is not our intention to dwell upon the numerous details collected in our studies of the life cycles of the different organisms. Though the broad types of growth are the same with all, the morphological details, of course, differ considerably. Figures 25 to 30 (Pl. E), 31 to 36 (Pl. F), and 37 to 40 (Pl. G) will furnish sufficient proof in this direction, especially when compared with our illustrations of *B. azotobacter*. It may suffice to add the following remarks:

Figures 25 to 27 (Pl. E) illustrate the appearance of the same cultures of *B. subtilis* on a beef-agar slant, made from a 1-day-old colony on a beef-agar plate. The smear made directly from the colony showed

the typical spore-forming rods as reproduced in the atlas of Lehmann and Neumann (11, pl. 47, fig. V). Figure 25 (Pl. E) was made from a preparate from a 2-day-old agar slant. Spores dissolve into stage D; and thick-walled, more or less irregular regenerative bodies are being formed. This process is going on in the 6-day-old culture (fig. 26 of Pl. E). The "melting" of the spores is clearly visible. The regenerative bodies have increased in number as well as in size. Some forms resemble very much those of *B. radicicola*. After eight days (fig. 27 of Pl. E) these regenerative bodies are either dissolved entirely into a readily stainable symplasm or they produce bright granulated spores (type H), which later also pass over into the symplastic stage. Sometimes the unstainable content of the regenerative bodies slips out of the dark-stained cell wall, forming an unstainable symplasm like that frequently produced by cells of the C type of *B. azotobacter* (fig. 6 of Pl. A; fig. 7 of Pl. B. See also the mixture of stained and unstained symplasm in fig. 19, Pl. D). The new set of regenerative bodies developing from the symplasm, especially from the dark-stained material derived from those irregular forms, usually showed rodlike forms stretching out into long granulated threads, which, in their turn, divided themselves into the normal spore-forming rods typical of *B. subtilis*.

This behavior was observed, only slightly modified, with all cultures of spore-forming rods. Figure 28 (Pl. E) shows this regerneration of the new threads from the symplastic stage as it was found in a 2-day-old transfer of the "yellow bacillus" (No. 41), made from a 12-day-old peptone-glycerin solution into the same liquid substrate. The thread on the right side of the field illustrates the situation especially well. As the upper part is broken off, the gonidia inside the cell, which caused the formation of the short branch on the lower part, become visible. The symplasm still contains several regenerative units which apparently are checked by the vigorous absorptive action of the thread.

The special appearance of many types of growth of *Bact. bulgaricum* is plainly discernible in figure 29 (Pl. E), made from a 6-day-old stab culture in yeast-whey agar. Large and small rods (types L and F), pointed forms (type M), the formation of regenerative bodies (type I) budding (type K) are clearly visible. On the left side of the figure two long, thin threads grow ("branch" or "bud") out of the same point in a larger rod. Below this another thick rod, showing granular decomposition (type C), is reproduced. In the middle of the field some thin, pale symplasm (D) is spread out. Above, a thin pale thread containing darker stained bodies (type G) crosses the field. Some small cells of type E are lying close to it. That the round cells budding out of the rods are indeed regenerative bodies is proved by their germination, the new rods growing out in one or in two directions.. This frequently happens when the regenerative bodies are still connected with their mother cell.

The formation of the symplasm in an 11-day-old ammonium-citrate-glycerin solution of *B. fluorescens* is shown in figure 30 (Pl. E). This figure should be compared with figure 11 (Pl. B), showing the formation of stained symplasm of *B. azotobacter*. The dark rods also visible in the former figure are of the H type.

In figure 31 (Pl. F) cells of *Sarcina flava* from a 1-day-old beef-agar slant are reproduced partially disintegrating into the symplastic stage. The small symplasm in the center has already entered the formation of regenerative units. Many of the cells are in the conjunct stage.

Figure 32 (Pl. F) illustrates the transformation of the symplasm of *Streptococcus lactis* into many normal forms and some round regenerative bodies, as observed in a 5-day-old peptone-lactose solution. As far as this transformation has already taken place, it is clearly discernible that indeed, as mentioned before, the whole material is used again for the reproduction of new cells practically without leaving any remnants. Figure 33 (Pl. F) shows another "flake" of symplasm of *Streptococcus lactis* from a 3-day-old milk culture containing many regenerative units and some globular regenerative bodies. This illustration is of special interest for the following reasons: Such globular bodies of different diameters are produced by all kinds of bacteria (cf. fig. 13 of Pl. C; fig. 25, 26, 27 of Pl. E). If they are dispersed in their symplasm and this is embedded in the equally deeply stained casein of the milk, it looks nearly as if the albuminous substances of the milk were forming granules which later produce normal bacteria by germinating or stretching. The center of figure 33 (Pl. F), where a rather compact symplasm is lying above a very thin film of casein, shows that these things are entirely separate and different. However, in the lower left part of the field some symplasm is embedded in a thicker layer of casein, and here the situation is much less clear. Now, Fokker (5), one of the few authors who are still fighting in favor of spontaneous generation, has repeatedly pointed out that his standpoint is strongly supported by the fact that the albuminous substances in animal tissues, as well as in milk and in blood, produce small granules which later develop into normal bacteria. The assumption that his subtrates were not sterile, of course, does not furnish a complete explanation of these peculiar observations. We believe, however, that our discovery of the symplasm and of its regenerative units settles this question.

That the formation of the symplasm and the regeneration of new cells are by no means an abnormal occurrence merely caused by the unnatural conditions under which our cultures are compelled to live in the laboratories can be deduced without great difficulty from different facts already mentioned. However, we thought it useful to add to the illustration of the milk culture another one reproducing an entirely "natural" occurrence. Figure 34 (Pl. F) was taken from a smear made directly from the content of a root nodule of red clover. The irregular, frequently branched

large forms[1] are passing over into the symplastic stage. Many bright gonidia, some deeply stained regenerative bodies, and a few normal slender rods are seen. This illustration should be compared with figures 26 and 27 of Plate E.

The two salt-water spirilla included in our experiments were also inclined to produce symplasm, globular and irregular regenerative bodies like the representatives of all other groups of bacteria. We have preferred, however, to show in figures 35 and 36 (Pl. F), which were made from a salt-beef-agar slant only 4 hours old, some facts which confirm and explain two observations made several decades ago. In 1887 Sorokin (19) published a preliminary communication upon his "new species" *Spirillum endoparagogicum*, of which, so far as we know, a full description has never been given. His illustrations, reproduced in several textbooks, show clearly that he also found a budding bacterium without becoming aware of this fact. That the bright granules contained in the large spirilla and budding out of it, forming new small rods and spirilla, were not endospores, as the author asserts, seems to be beyond question. No test was made of their heat resistance, and in our opinion the fact that many of them were produced in the same cell proves sufficiently that they were gonidia. Their globular form is also much more in agreement with this opinion than with the assumption that they were endospores. Figure 35 (Pl. F) shows the same budding of our salt-water spirillum. Many of the irregular "involution" forms, so frequently observed with other spirilla, belong also to this type of growth. Furthermore, in figure 35 (Pl. F), as well as in figure 36 (Pl. F), several round regenerative bodies are reproduced, some of them being in the germinating stage. They are either dark-stained like those of other bacteria or they remain unstained when treated with aqueous anilin dyes. If such unstained forms are budding out of the end of a spirillum, as can be seen in the center and at the right side of figure 35 (Pl. F), we have apparently before us the same occurrence which was described by Prazmowski in 1880 (17, p. 43). We have not yet tested the heat resistance of these bodies. It is possible that they are parallel forms of the exospores found in the spore-forming L type of *B. azotobacter*. In the meantime they may be registered as unstained regenerative bodies. Some different types of germination are exhibited by the three regenerative bodies in figure 36 (Pl. F). The lower right part of figure 35 (Pl. F) contains several spirilla which may be in the conjunct stage. They are wound closely around each other, forming apparently one thick cell, only the end parts being separated. An analogous occurrence with *Spirochaeta obermeieri* has been recently observed by Levy

[1] They have been called "bacteroids" by Brunchorst because this author conceived the wrong idea that they were not bacteria, but cell products looking somewhat like bacteria. We are unable to understand how such an entirely incorrect term can still be used in modern scientific publications.

(12), who is also of the opinion that some "copulative" process takes place.

In future publications we will have to give more illustrations showing the different forms of the various kinds of regenerative bodies produced either directly by the different bacteria or by their symplasms. It seems as if such irregular, sometimes monstrous-looking formations as reproduced, for example, in figure 26 (Pl. E), are very constant and very characteristic for the species to which they belong. This is already well known in the case of *B. radicicola*, and our figures 37 and 38 of Plate G may demonstrate the same fact in relation to *Micrococcus candicans*. The preparates were made from 6-day-old cultures in an ammonium-citrate solution. One strain (No. 44) had been isolated about six years ago from Russian black soil; the other (No. 45) nine years ago from evaporated milk. The characteristic appearance, as well as the uniformity of both illustrations, deserves our full attention.

Figures 39 and 40 (Pl. G) show the formation of well-stained gonidia by the yellow bacillus (No. 41) and by *B. fluorescens* (No. 40). In both cases the transformation of gonidia into regenerative bodies is clearly visible (cf. fig. 29 of Pl. E). Figure 39 (Pl. G) contains also in its lower left quarter some germinating regenerative bodies. The "budding" process is very conspicuous in this case, but the second picture makes it clear that the size of the gonidia frequently becomes so small that even a very careful observation of the stained preparate is hardly of any use. Besides, as many of these gonidia do not take the stain at all, some filtering experiments and the observation in the dark field seemed to be preferable.

## FORMATION OF FILTERABLE GONIDIA

The formation of gonidia has been observed with all our different cultures, but whenever we saw a large number of gonidia in our preparates, there were always some, frequently many, just at the limit of visibility. It was to be expected that these would pass through Chamberland bougies. As our other experiments had shown that these gonidia are indeed living entities, some tests seemed to be of interest, especially in view of the many open questions concerning the occurrence and character of filterable vira.

The following cultures were used for making filtering tests:

No. 1 (*B. azotobacter*), 24 days old in a mannite-nitrate solution; No. 31 (*B. subtilis*), 11 days old, in ammonium-citrate-glycerin solution; No. 33 (*Tyrothrix tenuis*), No. 35 (*Bact. pneumoniae*), and No. 40 (*Bact. fluorescens*), each from a 2-day-old culture in ammonium-citrate-glycerin solution.

The filtrates were first tested under the microscope. Stained preparates had to show the absence of large forms. By the use of the dark field the small gonidia could easily be seen, some of them being actively

motile. These filtrates were then transferred to beef agar, beef broth, milk and blood serum. After incubating, the macroscopical appearance was the same in all cases. On the agar slope, especially on its lower moist part, a very scant, thin, slimy growth, somewhat resembling a very thin layer of small droplets of dew, became visible. A stained preparate from a 4-day-old slant clearly showed the gonidia germinating to minute rods (fig. 41 of Pl. G). The other substrates also gave no conspicuous growth.

The dark field proved more efficient in observing these almost invisible forms. Figure 42 (Pl. G), made from the same 4-day-old slant as figure 41, shows clearly that the filterable gonidia also form a symplasm in the same manner as the larger ones, which, in its turn, produces new small cells. In order to bring out more definitely the structure of this symplasm, it was necessary to make a very dark print, thereby obliterating several free granules which were also visible in the field.

These facts are in good agreement with the observations of various authors concerning filterable vira. In one of the latest publications along these lines Healy and Gott (9) described the filterable organism causing hog cholera as small globules or rods (0.2 to 0.3$\mu$) when growing on ordinary media appearing in slimy clumps which are either well stained with aqueous dyes or are not stained at all.

As a regeneration of larger forms could in no case be observed on the subtrates mentioned, we also transferred small quantities of filtrates of cultures 31, 35, and 40 into ammonium-citrate solution. Here a quick regeneration took place. After two days some sediment was already formed, which after shaking caused a distinct turbidity of the solution. Under the microscope in the stained preparate many pale, stained, small granules and minute rods were visible, as before, and also larger dark stained oval forms 0.5 to 1$\mu$ broad, 0.75 to 1.5$\mu$ long. These forms still differ considerably in their appearance from the normal rods of *B. subtilis* or *Bact. fluorescens*. They may be classified as regenerative bodies. That they will turn back entirely to the normal large vegetative cells is not doubtful, but this still remains to be tested experimentally. So far our tests have been repeated three times with *Bact. fluorescens* and twice with *B. subtilis*. The results were identical. For the filtration we used three different filters, which were controlled in each case by obtaining sterile filtrates from 1-day-old cultures of *B. subtilis* or *B. azotobacter* 1.

In carrying out experiments like these, however, another possibility of obtaining erroneous results must be kept in mind. Not only must the media be carefully prepared and sterilized but all glassware must be thoroughly treated with some cleaning fluid such as chromic acid, which destroys entirely all bacterial forms. The fact that mere sterilization is not sufficient is shown by the following test:

Particles of symplasm containing many regenerative bodies were carried from a mannite-nitrate solution to a similar medium and heated

in the autoclave for half an hour at 20 pounds' pressure. Even after this harsh treatment the microscopical picture was practically unchanged. As substrates rich in organic matter, such as beef agar, frequently contain symplasm and regenerative bodies resulting from former bacterial growth, they are especially liable to give misleading results.

## DISCUSSION OF RESULTS

We hope that the facts mentioned in this preliminary communication will suffice to awake an adequate interest among our fellow bacteriologists, as there are numerous problems which now can be attacked successfully from this new standpoint. It is true that several authors before us have already spoken of the "life cycles" of bacteria. In most cases, however, they meant only the straightforward (not "cyclic") development, consisting in stretching and dividing of the cells, sometimes combined with the formation and germination of endospores. Fuhrmann (7, 8), who also wrote upon the "*Entwicklungskreise*" of bacteria, made some correct observations concerning the formation and further development of the gonidia. He was wrong, however, in concluding that these "granula" which he found in some spore-free bacteria were practically counterparts to the endospores in the "life cycle" of the spore-forming bacilli, and his opinion upon the "detritus" resulting from the disintegrating cells—namely, the symplasm—was far from being correct. In this direction Fokker (5) came much closer to the truth. It is not impossible, of course, that by a thorough sifting of the literature we shall discover some entirely forgotten author who was already on the right track. So far as we know now, only one bacteriologist has previously seen all the different stages of growth typical of the full life cycle of the bacteria. We refer to De Negri's important "*Untersuchungen zur Kenntnis der Corynebacterien*" (16), which appeared this spring, when we had just begun to prepare this paper for publication. A comparative study of the illustrations of his article and those of the present paper will be very instructive. He registered the following forms produced by the organism which causes the "malignous granulom:"

Large globules (2.5 to 5.5$\mu$) sometimes in sarcina form, eventually developing round or rodlike germs or buds ..... Our types A and B
Large forms containing granules occasionally unstainable ..... Our type C
Crumbly agglomerations formed by large forms "melting" together, which later give birth to new small forms ..... Our type D
Small globules frequently in chains ..... Our type E
Small short rods (¾ by 1$\mu$), small slender rods (¾ by 1½ to 2$\mu$), rods containing granules, curved rods, and rods showing racket form ..... Our type F
Granulated threads dissolving into small globules ..... Our type G
Entirely unstained bright rods ..... Our type H

Globular forms of different size sometimes showing a thin protruding rodlike form, irregular curved or clublike forms which later produce normal rods.....Our type I
Budding large globules, budding and branching rods and threads.....Our type K
Large rods and threads.....Our type L
Pointed rods containing large granules.....Our type M

This complete agreement is indeed very interesting, and as we ourselves have not worked with any representative of this group of organisms, De Negri's observations furnish a very welcome extension and confirmation of our statements concerning the life cycles of all bacteria. De Negri himself unfortunately failed to see that he was touching this general problem. He confined his studies almost exclusively to those corynebacteria causing "malignous granulom" and to some closely related forms. Therefore he was carried away to the entirely incorrect conclusion that those large budding forms were some kind of "blastomycetes," and the organism studied by him should be separated from the bacteria and placed among the Fungi Imperfecti. A comparative study of any of the common bacteria—for example, *B. subtilis*—would easily have prevented this serious error.

For *diagnostic* and *systematic* purposes a full knowledge of the life cycles of the bacteria will naturally be of the greatest importance. In our opinion the following morphological details should be studied in every case.

1. Vegetative cells; formation and germination of spores
   - Spore-free and spore-bearing cells
   - Arthrospores, formation and germination
   - Endospores, formation and germination
   - Exospores, formation and germination
2. Conjunction of different cell types
3. Gonidia, formation and development
   - Budding, liberating, germination, development in toto to regenerative bodies, to exospores, or to full-sized cells
4. Symplasm, formation by
   - Spore-free cells
   - Spore-bearing cells
   - Arthrospores
   - Endospores and exospores
   - Regenerative bodies
   - Gonidia
5. Regenerative bodies
   - Formation by
     - Spore-free and by spore-bearing cells
     - Arthrospores, endospores, and exospores
     - Gonidia of different types
     - Symplasm of different origin
   - Germination of the different types
   - Development in toto to vegetative cells or to spores

The improvement of the present situation is obvious. As the full life cycle of probably every species of bacteria can be studied without difficulty within a few weeks, provided suitable media are known and used for the experiment, we may hope that the time of reckless species-making will soon be ended. As said before, "good" species will win very much by such renewed and thorough study. The innumerable others, however, will have to take their modest place as links in those life cycles to which they really belong, or they will have to be canceled entirely. That the discovery of the conjunct and symplastic stage and further experimental studies upon it are of fundamental importance for reaching correct conclusions concerning species or varieties is beyond question.

Undoubtedly all our *physiological* studies will gain in much needed conformity and accuracy when established on the new broad morphological basis. It is to be hoped that such investigations now will also meet with more interest in botanical laboratories, where many of the general problems in bacteriology should be studied, as usually the time of agricultural and medical bacteriologists is completely taken up by their more specialized work. For instance, those curious but heretofore entirely unexplainable regular seasonal variations in the activity of bacteria in soils, quite frequently observed in Europe as well as in America during the last years, now seem to become explainable as a result of the seasonal effect upon the different modes of multiplication and propagation of the bacteria. A similar dependency on this factor then would exist as with other organisms. At least we can hardly consider it being merely an accidental coincidence that essentially the same annual curve, showing a maximum in spring and another one in autumn, is also followed by lower fresh-water algæ, where, as Transeau's careful investigations (20) have shown, the temporary prevalence of spore formation and of vegetative processes apparently represents the principal cause of these variations.

Concerning *pathological* problems, we readily admit that we are entirely laymen. However, we feel sure that this branch of bacteriology also would win considerably by making use of our observations. They show that Henri's (10) very interesting results obtained with *B. anthracis* could easily be duplicated with this or other pathogenic species simply by studying the relation of virulence and type of growth. That those abnormal looking and abnormally reacting forms obtained by the French author by the application of ultra-violet rays are nothing else than some of the regular though heretofore unknown types of growth of *B. anthracis* needs hardly be emphasized. Investigations upon the relations existing between pathogenic and nonpathogenic bacteria, as well as the experimental transformation of one type into the other, now undoubtedly become much more accessible and promising. The same holds true concerning the filterable vira. At least some of them are surely to be explained as nothing else than filterable gonidia of well-known bacteria.

That the discovery of the complete life cycles of the bacteria solves also some problems in *general biology* has been indicated earlier in this paper, when Fokker's theory (5) concerning the development of bacteria from granules in milk or blood was discussed. It may be added that also those much doubted and disputed strange observations of Bastian (2, 3), so persistently and extensively defended by their discoverer, now are coming under entirely new aspects. Readers interested in this question should compare especially Plates IV and V of Bastian's "Nature and Origin of Living Matter" (2) and those on Plates X and XI of his "Evolution of Life" (3) with our illustrations of the different kinds of symplasm and regenerative bodies. Figure 33 of the last-named plate (XI) looks practically like a reproduction of our preparate shown in figure 14 (Pl. C). Bastian was wrong, of course, when he considered those large cells as being some torula form; but we know that De Negri (16) made the same mistake recently, which indeed is quite excusable. That the budding large cell in our figure 14 is really nothing else than a type of growth of a spore-forming bacillus will probably even now be doubted by one or the other bacteriologist. It is superfluous to point out that we do not share Bastian's ideas concerning abiogenesis. Our standpoint in this case is the same as in regard to Fokker's hypotheses. The weak points in Bastian's experiments are sufficiently clear to every expert reader of his books. This, however, should not lead to discarding indiscriminately all his undoubtedly carefully made microscopical observations.

It goes without saying that we will readily furnish subcultures of the strains used in our studies to everyone who asks for them. But it probably would be still more interesting and surprising to our fellow bacteriologists if they would make some investigations with their own well-known stock cultures along the lines discussed in the foregoing pages. Even a renewed microscopical study of old stained preparates may become very instructive. For example, the senior author also did not know that for more than 11 years he had in his collection, patiently waiting to be photographed, that fine preparate now shown in figure 6 (Pl. A) until, as stated before, he decided to take down his "theoretical blinders." We have already mentioned that a careful study of the illustrations contained in our daily used textbooks will now reveal several things which we were so very well trained not to see. Certainly the German philosopher Lichtenberg made a very wise remark when he said:

Was jederman für ausgemacht hält, verdient am meisten untersucht zu werden.

## SUMMARY

A comparative study of 42 strains of bacteria has shown that the life cycles of these organisms are not less complicated that those of other micro-organisms. As representatives of practically all groups of bacteria have been tested and all, without exception, behaved essentially in the

same manner, in all probability analogous results may be expected with all species of bacteria.

All bacteria studied live alternately in an organized and in an amorphous stage. The latter has been called the "symplastic" stage, because at this time the living matter previously inclosed in the separate cells undergoes a thorough mixing either by a complete disintegration of cell wall, as well as cell content, or by a "melting together" of the content of many cells which leave their empty cell walls behind them. In the first case a readily stainable, in the later case an unstainable "symplasm" is produced.

According to the different formation and quality of the symplasm the development of new individual cells from this stage follows various lines. In all cases at first "regenerative units" become visible. These increase in size, turning into "regenerative bodies," which later, either by germinating or by stretching, become cells of normal shape. In some cases the regenerative bodies also return temporarily into the symplastic stage.

Besides the formation of the symplasm, another mode of interaction between the plasmatic substances in bacteria cells has been observed, consisting of the direct union of two or more individual cells. This "conjunction" seems to be of no less general occurrence than the process first mentioned. The physiological significance remains to be studied.

All bacteria multiply not only by fission but also by the formation of "gonidia"; these usually become first regenerative bodies, or occasionally exospores. Sometimes the gonidia grow directly to full-sized cells. They, too, can enter the symplastic stage. The gonidia are either liberated by partial or complete dissolution of the cell wall or they develop while still united with their mother cell. In the latter case the cell wall either remains intact or it is pierced by the growing gonidia, which become either buds or branches.

Some of the gonidia are filterable. They also produce new bacteria either directly or after having entered the symplastic stage.

The life cycle of each species of bacteria studied is composed of several subcycles showing wide morphological and physiological differences. They are connected with each other by the symplastic stage. Direct changes from one subcycle into another occur, but they are rather rare exceptions. The transformation of spore-free into spore-forming bacteria seems to be dependent on the conditions acting upon the symplasm and regenerative bodies.

The discovery of the full life cycles of bacteria may be helpful in many directions. Systematic bacteriology now can be established on a firm experimental basis. Physiological studies will win considerably in conformity and accuracy when connected with morphological investigations along these new lines. Several problems in general biology are brought under more promising aspects. Agricultural bacteriology and medical also will derive much benefit.

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## PLATE A

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 1.—Azotobacter 11. Mannite-nitrate solution, 5 days old. Types A and L$\alpha$. Some cells in conjunction.

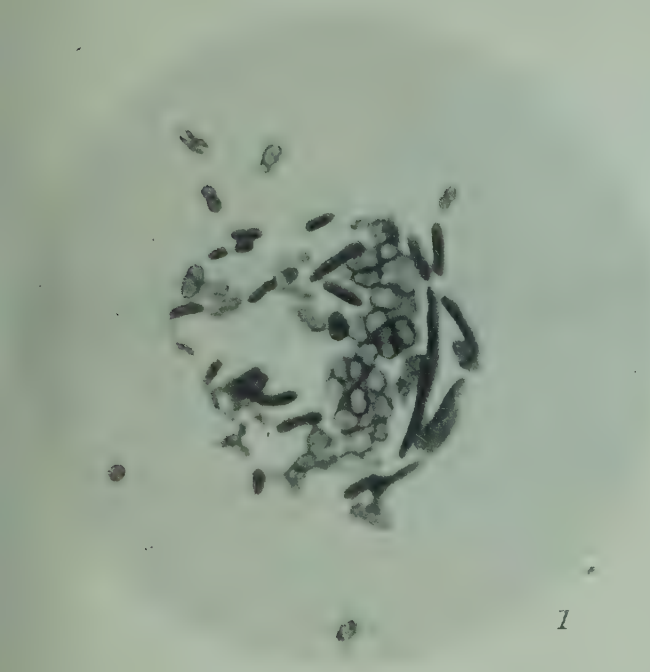
Fig. 2.—Azotobacter 21. Contact preparate from a colony on mannite agar, 4 days old. Types A, L. Most cells in conjunction.

Fig. 3.—Azotobacter 23. Contact preparate from a colony on mannite-agar, 4 days old. Types A, B, I, K$\alpha$, and many conjunct cells.

Fig. 4.—Azotobacter 13. Mannite-nitrate solution, 17 days old. Type K$\lambda$.

Fig. 5.—Azotobacter 14. Mannite-nitrate solution, 5 days old. Type B forming I.

Fig. 6.—Azotobacter 8. Beef bouillon. Type B forming types I and J.



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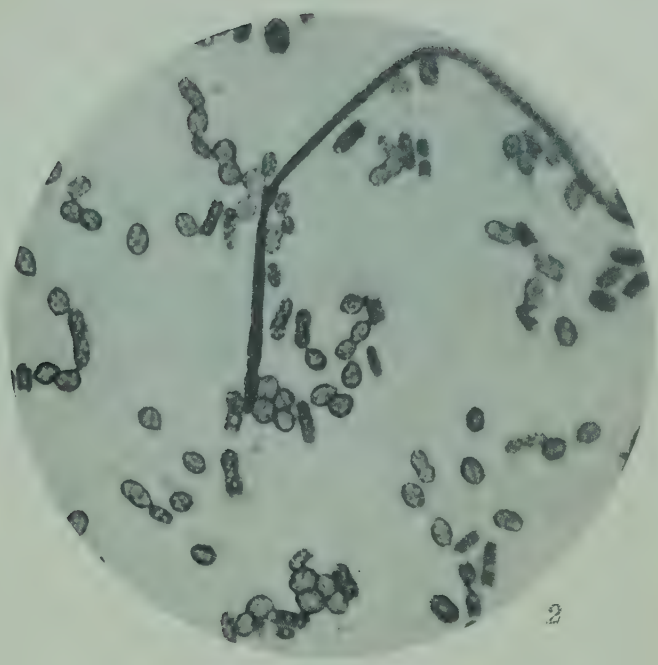

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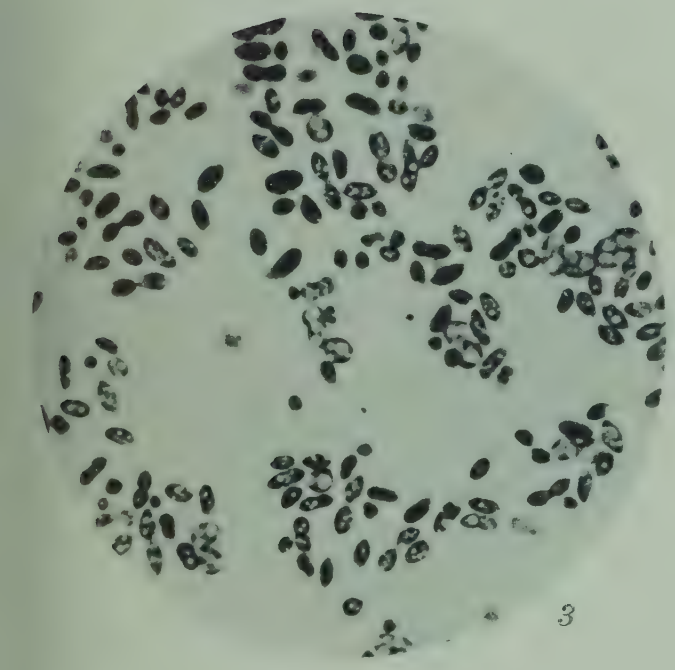

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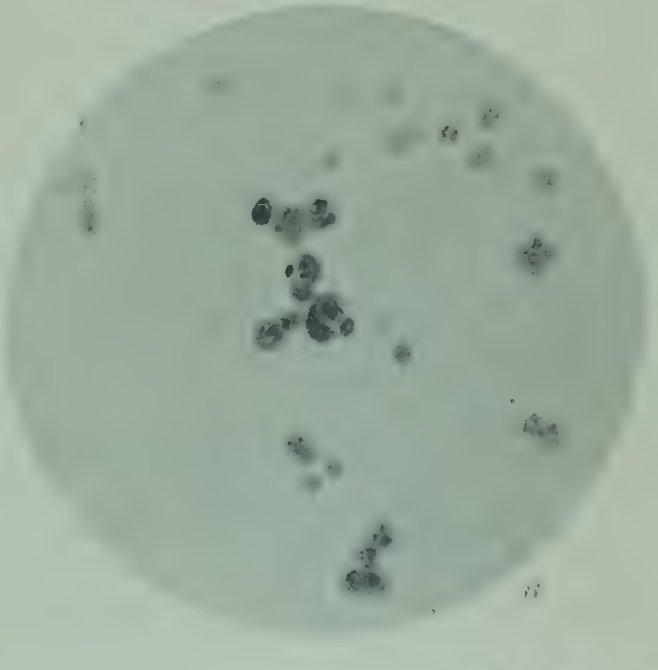

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## PLATE B

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 7.—Azotobacter 21. Mannite-agar colony, 4 days old. Type C forming types D and I.

Fig. 8.—Azotobacter 22. Mannite-agar colony, 4 days old. Type C forming D also A in conjunction.

Fig. 9.—Azotobacter 11. From a filter paper strip in mannite-peptone solution, 16 days old. Types A and B forming D.

Fig. 10.—Azotobacter 3. Mannite-peptone solution, 24 days old. Types L and M forming D.

Fig. 11.—Azotobacter 11. Mannite-peptone solution, 16 days old. Type D (stained) resulting from type C.

Fig. 12.—Azotobacter 6. From condensation water of mannite-agar slant, 1 day old. Type D (unstained) containing regenerative units.

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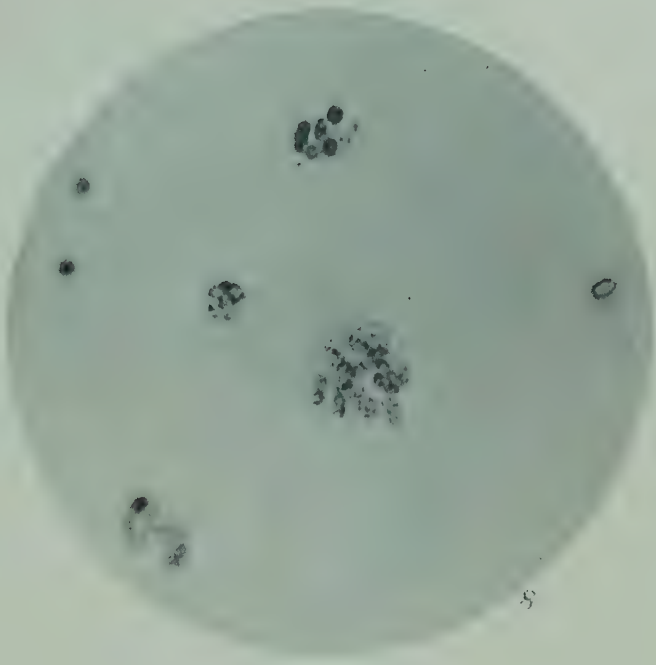

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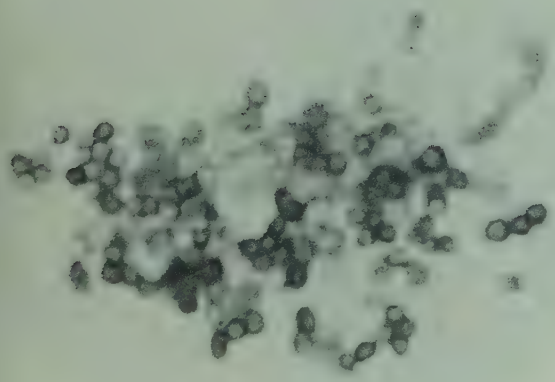

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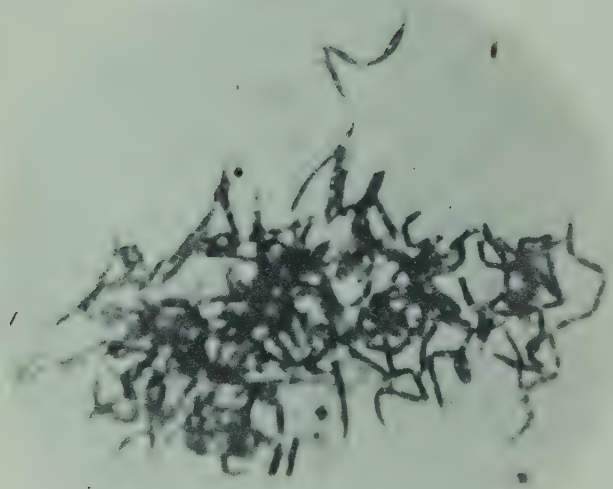

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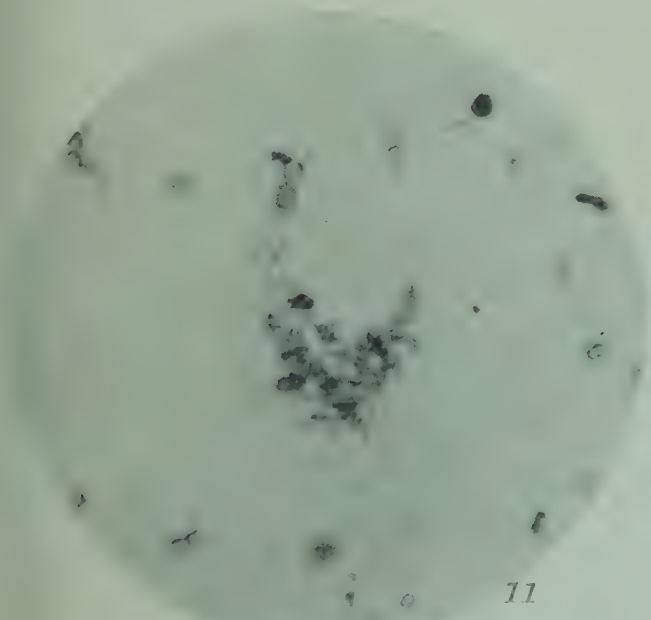

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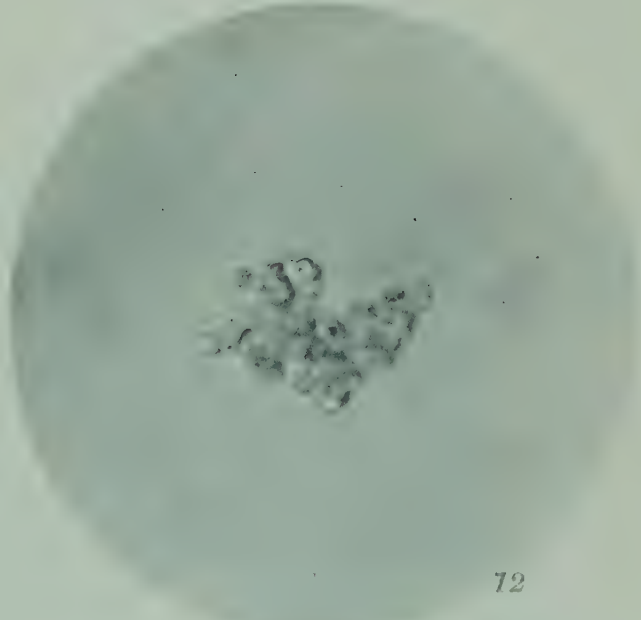

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## PLATE C

Magnification in all cases × 1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

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Fig. 13.—Azotobacter 24. Mannite-nitrate solution kept 5 days after having been heated 1 minute at 96° C. Types I and F developing from D. Some I germinating in conjunct stage and inclining to form spores.

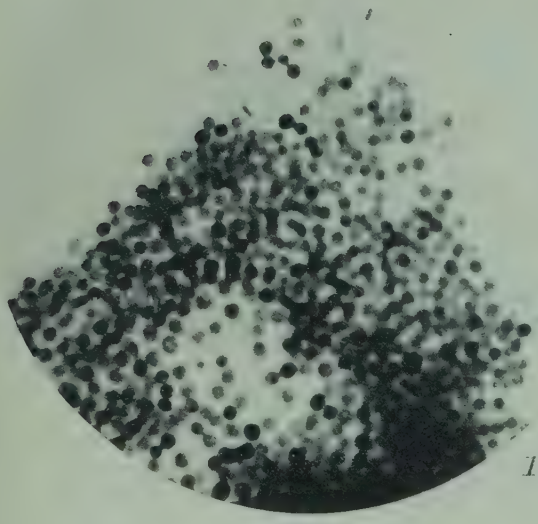
Fig. 14.—Azotobacter 1. Mannite-nitrate solution, 10 days old. Types B, K$\beta$, E, and F$\alpha$ developing from stained and unstained type D.

Fig. 15.—Azotobacter 15. From condensation water of a mannite-nitrate agar slant, 2 days old. Types F$\alpha$ and F$\beta$ developing from type D.

Fig. 16.—Azotobacter 17. Mannite-soil-extract agar, 2 months old. Types E, F$\alpha$, K$\phi$, and G developing from type D.

Fig. 17.—Azotobacter 17. Mannite-nitrate agar, 10 days old. Preparate treated with hot aqueous fuchsin. Type G, partially dissolved; also K$\phi$.

Fig. 18.—Azotobacter 7. Mannite-soil-extract solution, 14 days old. Type H forming D.



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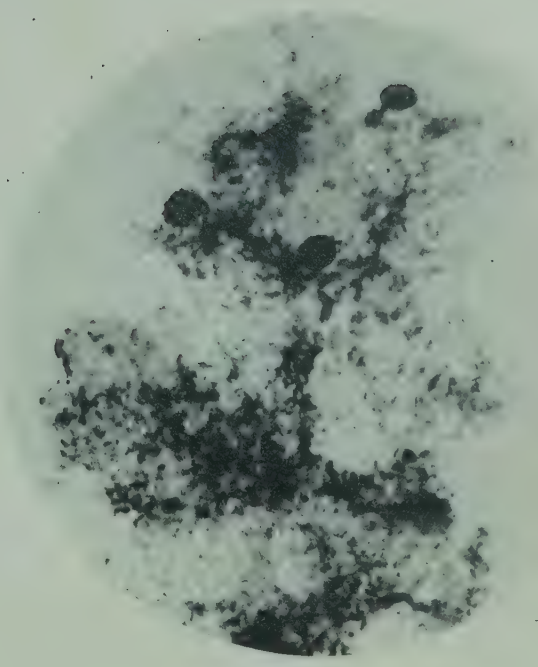

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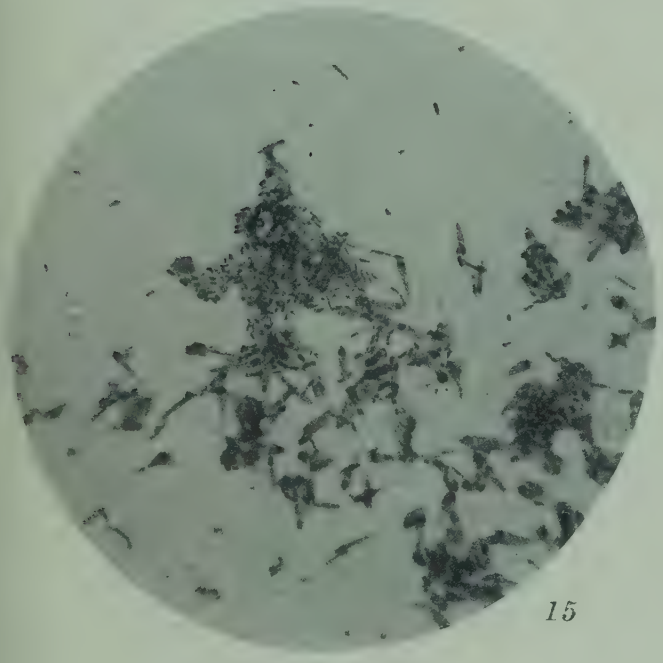

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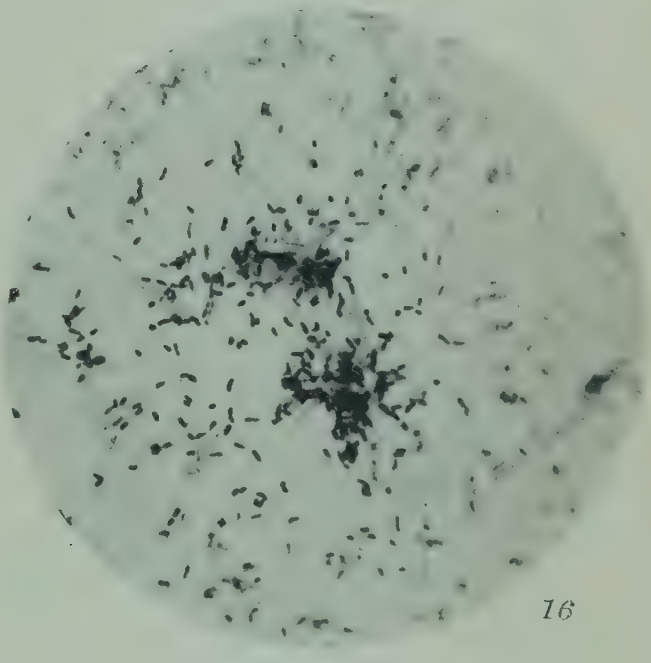

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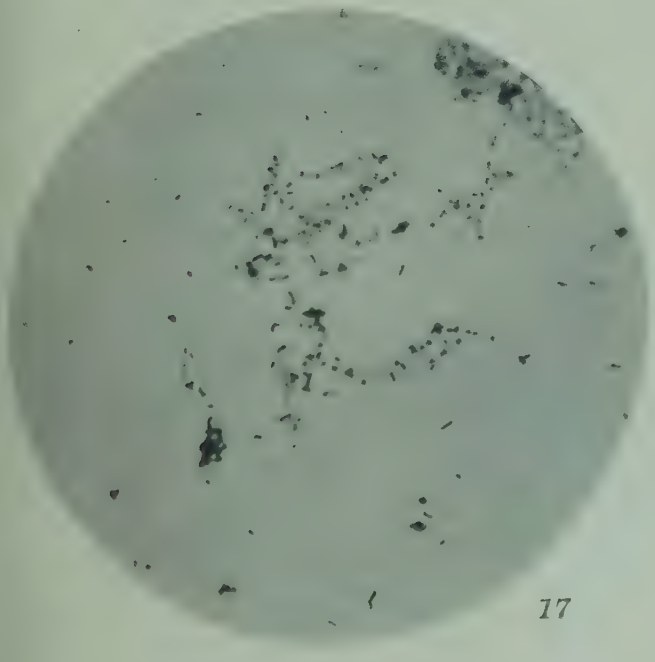

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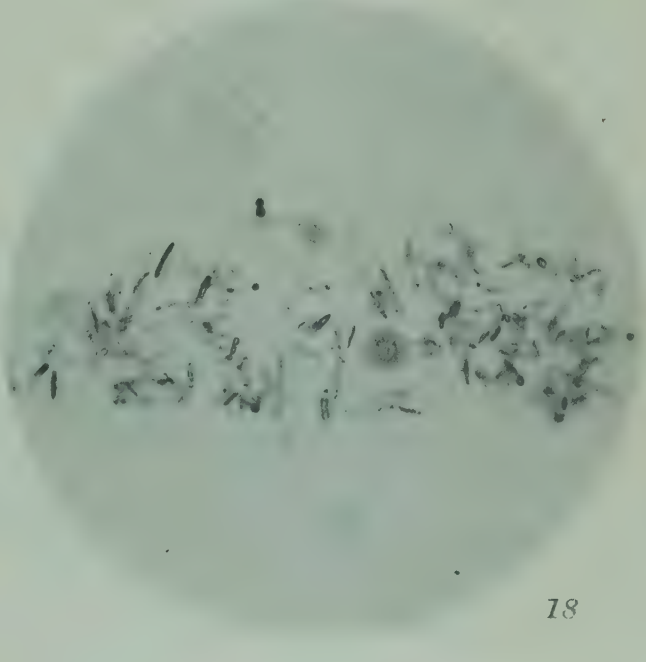

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## PLATE D

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 19.—Azotobacter 2. Mannite-nitrate agar, 23 days old. Spores forming type D.

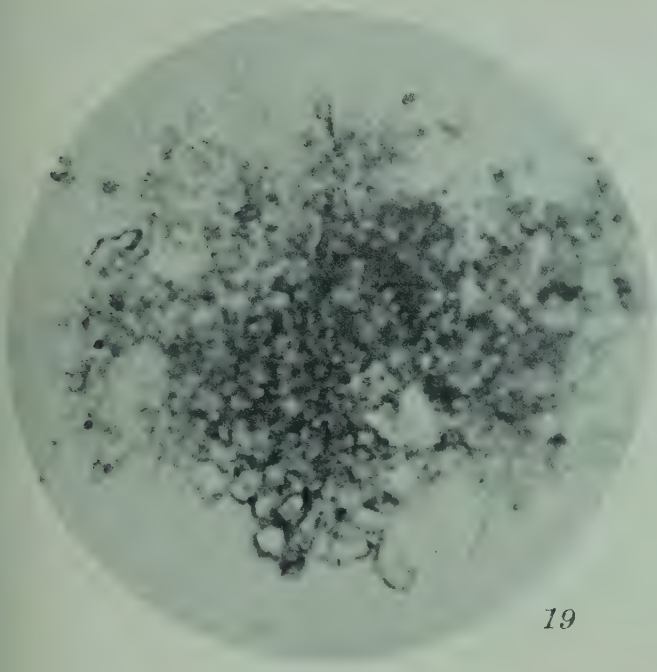
Fig. 20.—Azotobacter 2. Mannite-nitrate agar, 6 days old. Types L and F, endospores and exospores and dissolving of spores to type D.

Fig. 21.—Azotobacter 18. From a filter paper strip in mannite solution, 25 days old. Type L with gonidia, forming B (type Jλ).

Fig. 22.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Types E and F forming B.

Fig. 23.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Type B, formed by types E and F, germinating to type G.

Fig. 24.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Type Kγ.



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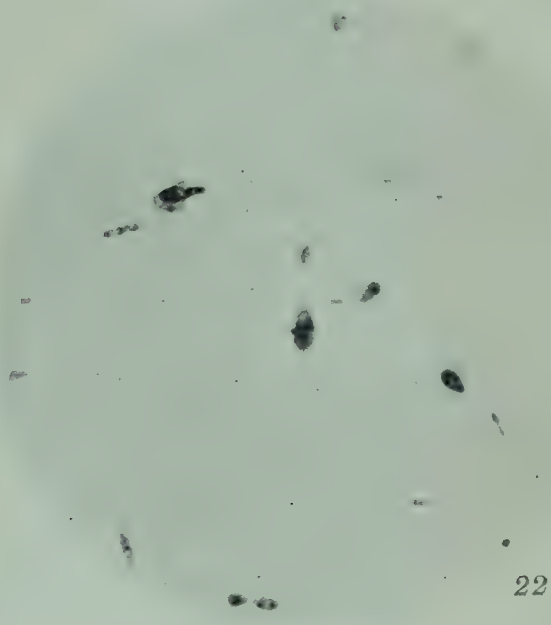

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## PLATE E

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 25.—*Bacillus subtilis* (No. 31). Beef agar, 2 days old. Types I and D formed by spores.

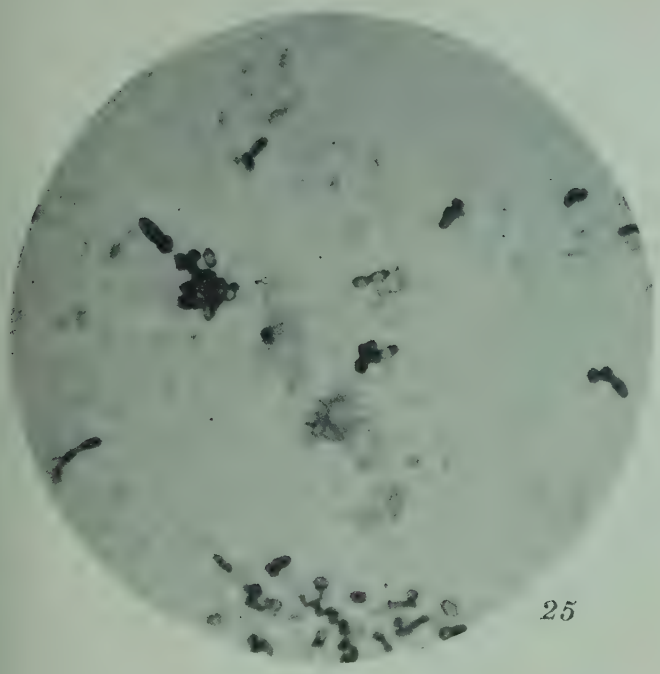
Fig. 26.—*Bacillus subtilis* (No. 31). Beef agar, 6 days old. Formation of type I.

Fig. 27.—*Bacillus subtilis* (No. 31). Beef agar, 8 days old. Type I forming H and stained D. Spores forming unstained type D.

Fig. 28.—Yellow bacillus (No. 41). Peptone-glycerin solution, 2 days old. Type I germinating from D, stretching to type L.

Fig. 29.—*Bacterium bulgaricum* (No. 49). Whey-yeast agar, 6 days old at 40° C. Types C, D, E, F, G, I, and K.

Fig. 30.—*Bacterium fluorescens* (No. 40). Ammonium-citrate-glycerin solution, 11 days old. Types D and H.



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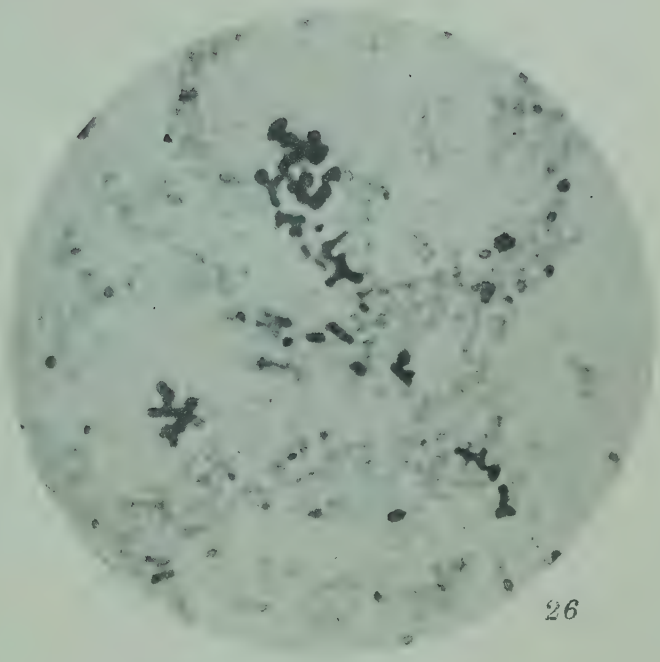

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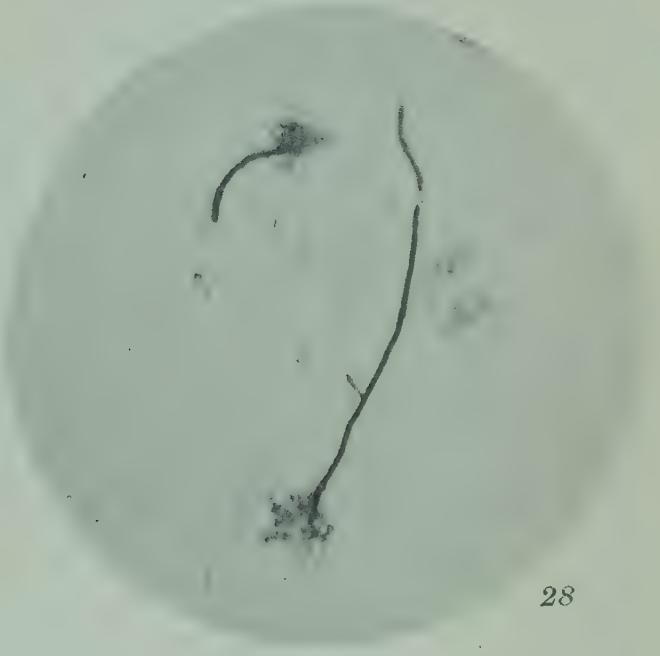

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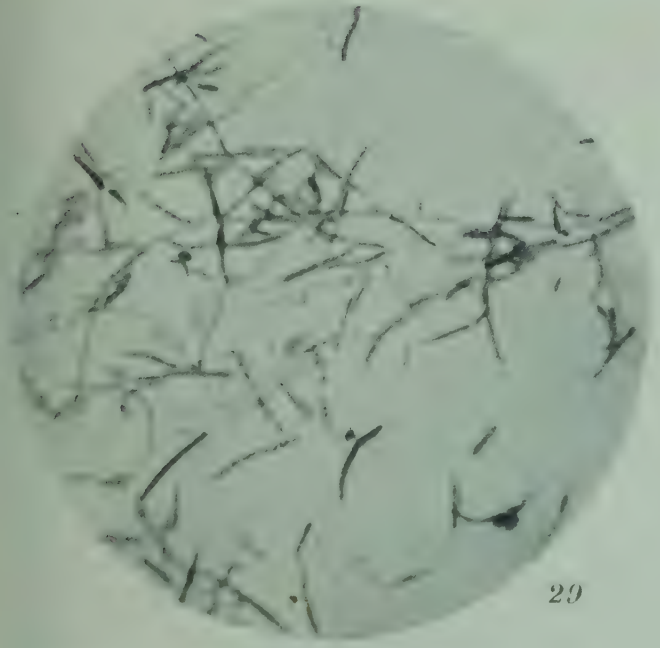

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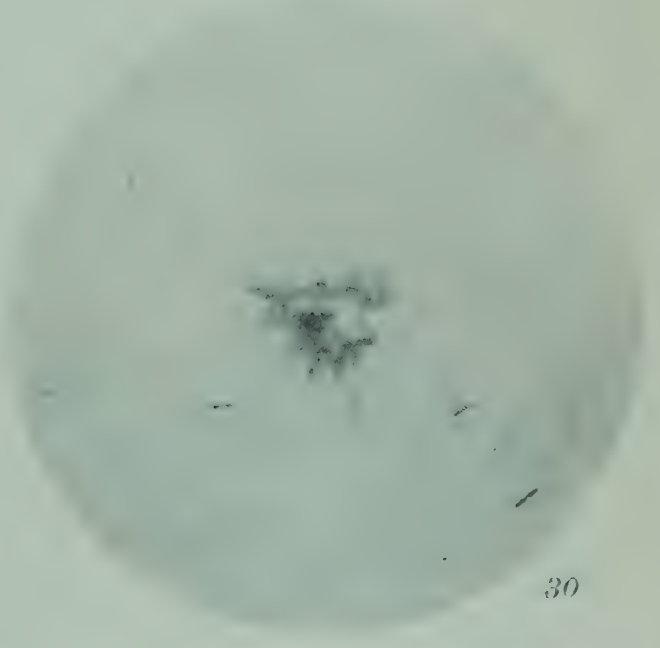

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## PLATE F

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 31.—*Sarcina flava* (No. 43). Beef agar, 1 day old. Type I in conjunction and forming D.

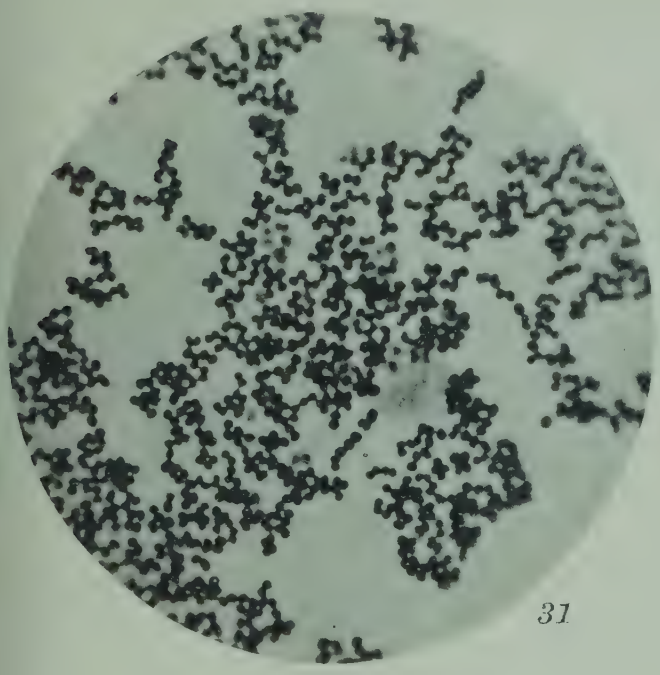
Fig. 32.—*Streptococcus lactis* (No. 48). Peptone lactose solution, 5 days old. Type D, with regenerative units, forming type I.

Fig. 33.—*Streptococcus lactis* (No. 48). Milk, 3 days old. Types D and I in casein.

Fig. 34.—*Bacillus radicicola* (No. 39). Types D and I. Preparate made from a root nodule in 1908.

Fig. 35.—*Spirillum* sp. from Great Salt Lake (No. 46). Beef broth plus 3 per cent of sodium chlorid, 14 days old. Budding and branching forms; stained and unstained regenerative bodies. Some cells in conjunction.

Fig. 36.—*Spirillum* sp. from Great Salt Lake (No. 46). Beef broth plus 3 per cent of sodium chlorid, 14 days old. Type I germinating.



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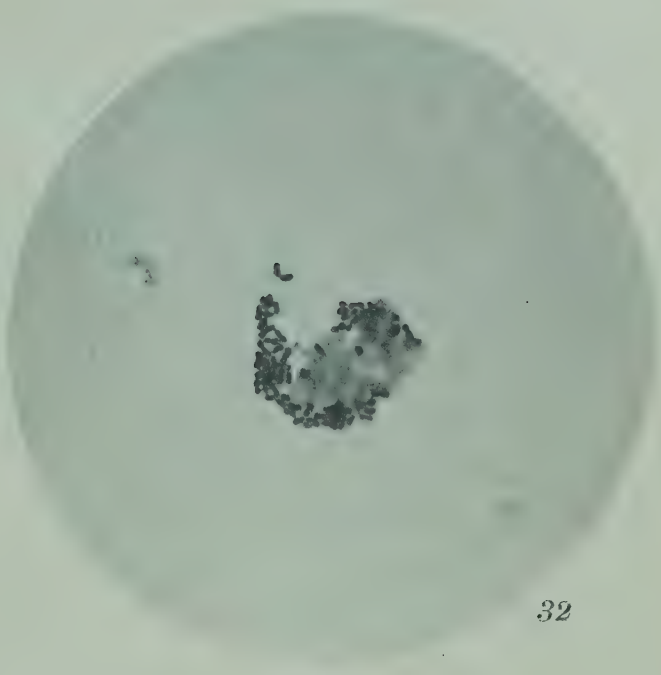

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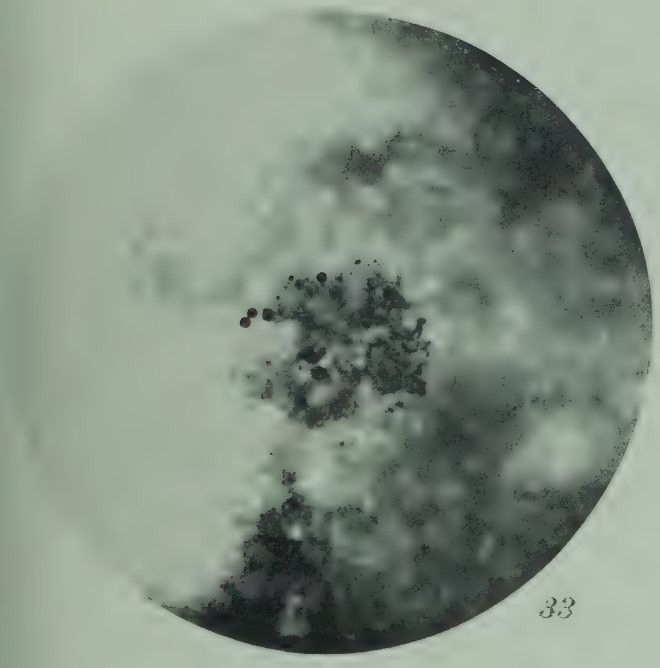

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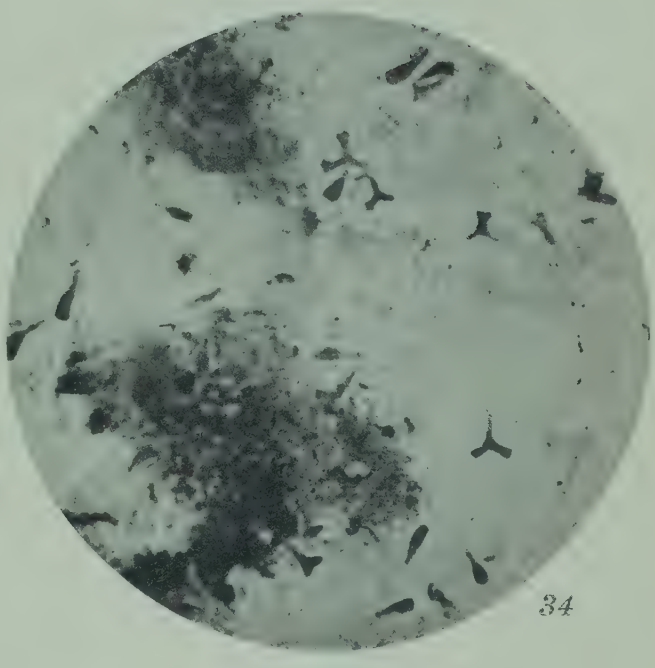

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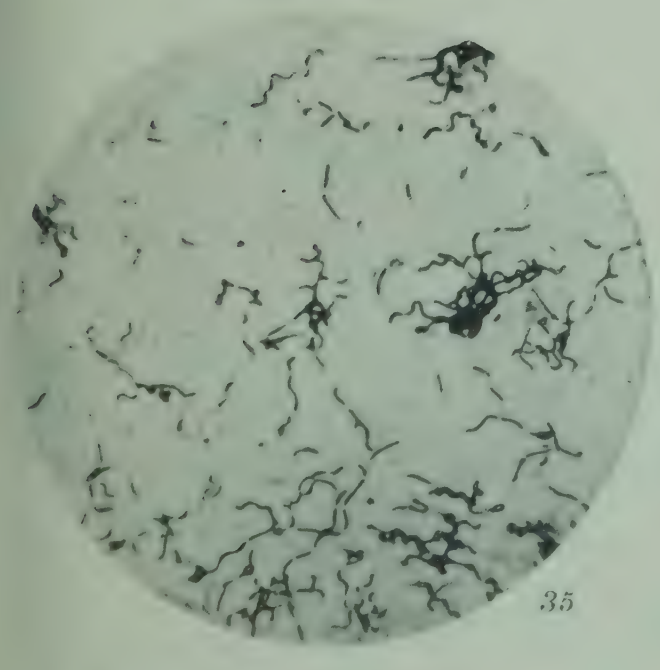

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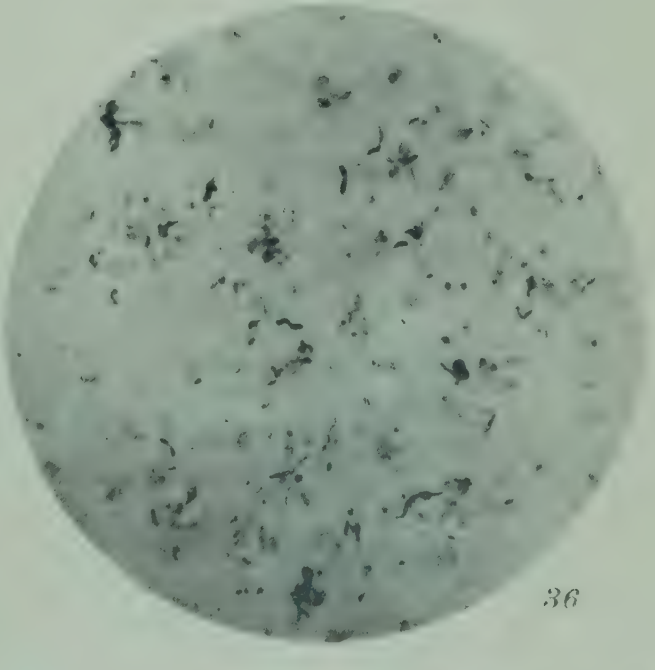

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## PLATE G

Magnification in all cases $\times 1{,}000$. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 37.—*Micrococcus candicans* from soil (No. 45). Ammonium-citrate-glycerin solution, 6 days old. Irregular, thick-walled type I.

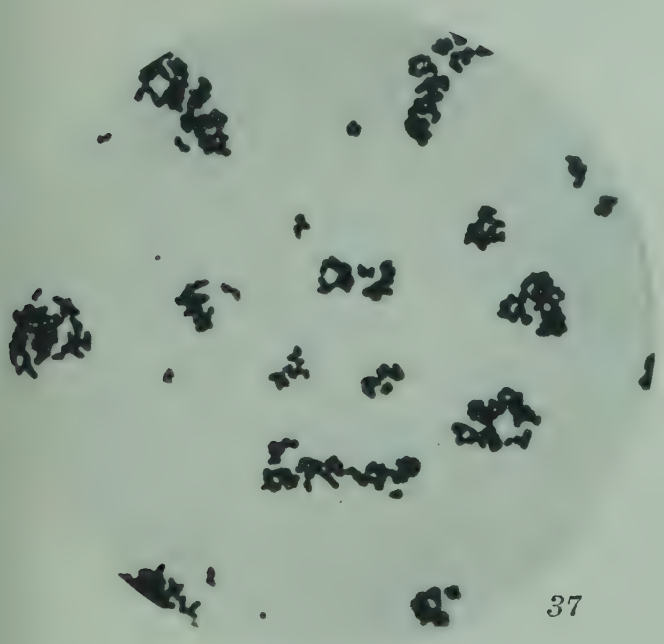
Fig. 38.—*Micrococcus candicans* from milk (No. 44). Ammonium-citrate-glycerin solution, 2 days old. Irregular, thick-walled type I.

Fig. 39.—Yellow bacillus (No. 41). Beef agar, 1 day old. Budding gonidia, formation and germination of type I.

Fig. 40.—*Bacterium fluorescens* (No. 40). Ammonium-citrate-glycerin solution, 2 days old. Budding gonidia, formation and germination of type I.

Fig. 41.—*Bacterium fluorescens* (No. 40). Beef agar, 4 days old. Filterable gonidia germinating.

Fig. 42.—*Bacterium fluorescens* (No. 40). Beef agar, 4 days old. Types D and F formed by filterable gonidia. Dark field.



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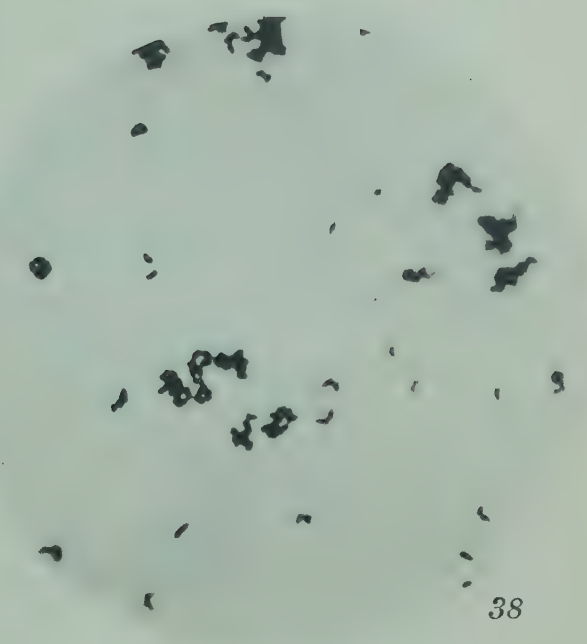

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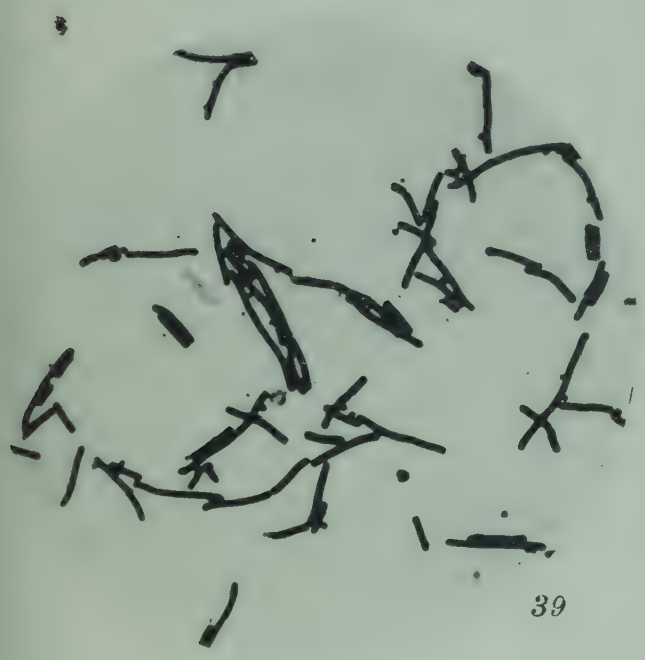

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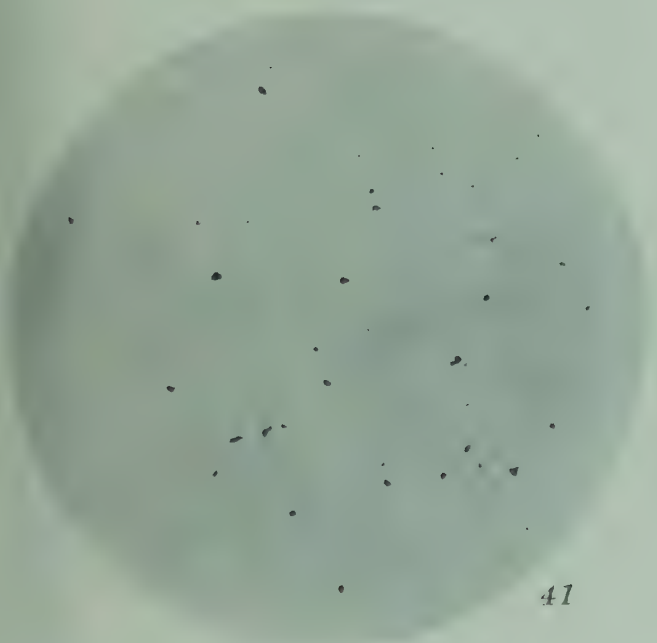

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# A RESPIRATION CALORIMETER, PARTLY AUTOMATIC, FOR THE STUDY OF METABOLIC ACTIVITY OF SMALL MAGNITUDE

By C. F. LANGWORTHY, *Chief*, and R. D. MILNER, *Assistant Chief, Office of Home Economics, States Relations Service*

## INTRODUCTION

A respiration calorimeter of the type of that described in a previous number of the JOURNAL OF AGRICULTURAL RESEARCH,[1] which is employed in the laboratory of the Office of Home Economics of the Department of Agriculture for the study of the metabolism of matter and energy in the human organism, is easily adapted to inquiries of similar character with other organisms. An apparatus much smaller than the one referred to has been developed in the same laboratory for use in the study of gaseous exchange and energy transformations of small magnitude and has been employed in investigations on the ripening of fruits and the wintering of bees. In fundamental principle this small respiration calorimeter is similar to the larger one mentioned above in that it combines a closed-circuit respiration apparatus and a continuous-flow water calorimeter. However, it differs from it in construction, having been modified in ways which make for ease of operation and for greater accuracy. Important changes have also been made in details, particularly with reference to its calorimetric features, the use of special devices for controlling and recording temperatures rendering it quite largely automatic in this respect. Brief accounts of this apparatus and of experimental work with it have been published,[2] but the details of construction and operation are given for the first time in the present article. A general view of the small respiration calorimeter is shown in Plate XCII.

## CONSTRUCTION OF THE RESPIRATION CHAMBER

The apparatus is devised so that chambers of different size or shape, constructed according to the varying needs of different investigations, can be substituted for each other. The chamber at present employed (Pl. XCIII) is 45 cm. square and 91 cm. deep, and has a total capacity of close to 185 liters. It was designed to accommodate a quantity of

[1] Langworthy, C. F., and Milner, R. D. An improved respiration calorimeter for use in experiments with man. *In* Jour. Agr. Research, v. 5, no. 8, p. 299–347, pl. 30–36. 1915.

[2] ——— ——— An improved form of respiration calorimeter for the study of problems of vegetable physiology. *In* Orig. Com. 8th Internat. Cong. Appl. Chem., v. 18, sect. viiic, p. 229–236, 1 pl. [1912.]

——— ——— A new respiration calorimeter for use in the study of problems of vegetable physiology. *In* U. S. Dept. Agr. Yearbook 1911, p. 491–504, pl. 65–67. 1912.

——— ——— Some results obtained in studying ripening bananas with the respiration calorimeter. *In* U. S. Dept. Agr. Yearbook 1912, p. 293–308. 1913.

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July 31, 1916
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fruit sufficient for experimental purposes when stored in it under conditions approximating those of commercial practice, or otherwise, as desired. For example, a large bunch of bananas may be suspended in it from a removable cross of iron pipe the ends of which rest upon cleats fastened in the corners of the chamber near the top. Other cleats at different levels provide supports for shelves or for trays, baskets, or other containers in which the experimental material may be placed.

The walls of the chamber, which are of sheet copper 0.5 mm. thick, are attached to the inner side of a framework of hard-maple strips, experience having shown that using wood in place of metal lessens the possibility of error. The vertical strip in each corner of the frame is 3 cm. square, with the inner corner cut away on each side to a depth of 5 mm. to form a recess for the corner of the copper walls. At the top and bottom of the chamber, and midway between, the ends of cross strips 25 mm. square are joined to the posts so as to form a rigid supporting structure which is strong, though consisting of but little material. At the lower end each post extends 4 cm. below the bottom cross strip, to provide a leg for the structure. Elbows of stiff sheet copper, with one branch soldered to the outer surface of the copper wall and the other screwed to the framework, hold the copper firmly in place against the wooden frame.

At the top of the chamber is a close-fitting removable cover (Pl. XCIII) of sheet copper on a maple frame, with the metal projecting in a rim to hold the cover in place. The edge of the rim is bent down to fit into a groove in the flange formed by extending the copper side walls at the top to the outer edge of the maple frame. Wax melted into the groove seals the joint between the top and side walls when the cover is in place.

In the middle of the upper half of each of two opposite sides of the chamber is a framed opening 13 by 18 cm., forming a recess in which a pane of glass may be sealed (Pl. XCIII). These windows afford a view of the contents of the chamber and opportunity to watch the changes taking place. They may also be arranged so that either one may be opened during an experiment to remove a sample of the material under observation, if desired.

Another wall has a circular opening framed with a tube 9.5 cm. in diameter, in which is fitted a device called an "outlet" (see Pl. XCIII) which provides apertures for pipes conducting a ventilating current of air into and out of the chamber, for resistance thermometers providing passage for water entering and leaving the heat absorbers, for wires leading to electric-resistance thermometers inside the chamber, and for other purposes, as needed, all of which may be sealed in place. All openings into the chamber other than the windows are thus brought together in the one device, which is easily separated from the chamber so that the latter may be removed and another of different capacity substituted for it. During an experiment every joint in the chamber is air-tight.

As part of the arrangement described on page 716 for preventing the passage of heat through the walls, ceiling, and floor of the chamber, these surfaces are duplicated by sheet-copper top, bottom, and sides screwed to the outer edge of the wooden frame, the inner and the outer metal walls being thus separated by an air space 25 mm. across. There are openings in these walls for the windows and the "outlet" described above.

Surrounding the entire chamber, about 25 mm. from the outer metal wall, is a heat-insulating cover (Pl. XCIII) consisting of two layers of cork board 38 mm. thick, alternating with three layers of museum board 6 mm. thick, built up on wooden frames. The top, bottom, and side sections are built separately, and the several sections fit together with double-rabbeted joints, so that any one may be removed without regard to the others, or the entire cover may be instantly taken off. One section, as shown in Plate XCIII, is divided along the vertical median line, and all pipes and wires passing to the copper walls and to the "outlet" are brought out between the two halves of this section. The sections covering the two sides have openings to correspond with those in the walls of the calorimeter. The bottom section of the cover rests upon a substantial oak platform raised about 18 cm. from the floor of the laboratory.

## DETERMINATION OF THE GASEOUS EXCHANGE

The respiration chamber in which the active material is confined is part of a closed air circuit through which a stream of air is constantly moving. The air which leaves the chamber is passed through purifying devices and returned again to the chamber. In the purifying devices the gaseous products resulting from the activity of the material in the chamber, which are carried out in the outgoing air, are absorbed. The purifying devices described below are those for the absorption of water vapor and carbon dioxid; but others could be substituted for these or connected with them if desired.

The quantities of water vapor and carbon dioxid carried from the chamber in a given period are shown by the changes in the weights of the absorbers during the period; and from these data, with due allowance for changes in the quantities of gases in the air of the chamber, the production of water vapor and carbon dioxid by the active material during the period is determined.

With a ventilation system of this type, as fast as any gas is removed from the air, other gas is introduced to maintain atmospheric pressure in the chamber. Usually oxygen is admitted, that being the gas consumed in respiration, as the term is commonly employed; but it is possible to vary the composition of the air at will, and if desired, to maintain an atmosphere of carbon dioxid or nitrogen or any other gas, which may be admitted to the system as oxygen is in the experiment as ordinarily conducted.

Oxygen to replace that consumed by the active material in the chamber is introduced into the air circuit from a reservoir of the gas. The quantity admitted is ascertained from the loss in weight of the container or by passing the gas through a meter. The amount admitted to the system and the change in the quantity of oxygen in the circulating air during a given period show the oxygen consumption of the material in the chamber.

A very light rubber bag on tne end of a small copper tube extending from the chamber affords some variability in the capacity of the system, and by thus allowing for changes in the volume of gas present resulting from lack of uniformity in the rates of absorption of gas from the circulating air and the admission of other gas to it, or from changes in the temperature of the air within the chamber or in barometric pressure without, serves as an air-tension equalizer.

### REMOVING WATER VAPOR AND CARBON DIOXID FROM THE AIR

The air withdrawn from the respiration chamber is forced first through sulphuric acid, which removes water vapor from it, and then through soda-lime, which removes carbon dioxid. The containers for the acid and the soda-lime, together with the air pump and the small electric motor by which it is actuated, are mounted on a stand with four shelves, called the absorber table (Pl. XCII). There are two parallel trains of absorbers on one shelf, one of which is in use while the units of the other are weighed and replenished.

The rotary air pump by which the circulation of air is maintained through the respiration chamber and the purifying devices has a capacity of approximately 100 c. c. per revolution, which is uniform for different rates of speed up to several hundred revolutions per minute. It is thus possible to vary the rate of ventilation of the chamber within a wide range simply by regulating the velocity of the pump, which is easily accomplished by means of a suitable rheostat to govern the speed of the motor which drives it. The rate of ventilation can be still further controlled, if desired, by means of a shunt in the air line between the inlet and outlet pipes of the air pump, with a valve to regulate the circulation through it. In the experiments for which the apparatus has thus far been used the former method has been sufficient, the pump being driven at a speed of 100 revolutions per minute, forcing 10 liters of air per minute through the system. With air ducts of brass pipe of 10-mm. bore the air flows in the circuit at very low pressure.

For absorbing water vapor from the circulating air, an acid bottle like that described for use with the large respiration calorimeter [1] but smaller in size has been found efficient. The bottle described by Williams,[2]

[1] Langworthy, C. F., and Milner, R. D. An improved respiration calorimeter for use in experiments with man. *In* Jour. Agr. Research, v. 5, no. 8, p. 306. 1915.

[2] Williams, H. B. Animal calorimetry. First paper. A small respiration calorimeter. *In* Jour. Biol. Chem., v. 12, no. 3, p. 323. 1912.

which is shown in Plate XCII, has also proved quite satisfactory. A charge of 500 c. c. of sulphuric acid in either of these bottles will continue for several hours to remove all water vapor from air passing through at any rate maintained in the experiments thus far conducted with the apparatus, even though in some cases water vapor in the air is near the saturation point. The bottle and the acid weigh less than 2 kgm., and by means of a sensitive balance of 10 kgm. capacity the change in weight during a given period is ascertained to an accuracy of 0.05 gm.

Carbon dioxid is removed from the air which leaves the acid bottle by soda-lime in a large-sized U-tube of special design (Pl. XCII). Each arm of the U consists of glass tubing 23 cm. long and 75 mm. in diameter, and the two are joined at the bottom by glass tubing of 15-mm. bore bent in a semicircle, leaving a narrow space between them. The upper end of each arm of the U-tube is closed by a ground-glass stopper from the top of which projects a glass tube of 10-mm. bore bent at right angles. The bottom of the stopper is closed except for an aperture of 10 mm., and in the space within the stopper is cotton wool to prevent particles of soda-lime from leaving the tube in the outgoing air.

A piece of fine-mesh brass wire gauze is put on the bottom of each large tube to keep the bent portion empty, and each arm is then filled to the stopper with soda-lime in particles of about the size of a dried pea, approximately 2 kgm. of soda-lime being required to fill both arms. This amount of material when fresh will commonly absorb at least 100 gm. of carbon dioxid before it needs attention, which is indicated by the color of the soda-lime. The gray-colored material, which is somewhat moist in the fresh condition, becomes white with use, owing to both loss of moisture and absorption of carbon dioxid. When the moisture is entirely gone the efficiency of the soda-lime is low; but by passing moist air through the tube it may be restored to such an extent that the soda-lime may be used for at least one more period.

To catch the moisture given up by the soda-lime to the dry air coming from the first water absorber, the air leaving the U-tube is passed through another bottle of acid. Both the acid bottle and the U-tube, for which there is easily room on the pan of the balance by which the gain in weight of the absorbers is determined, are weighed together to find the quantity of carbon dioxid removed from the circulation of air. Their total weight is less than 5 kgm., and their change in weight is ascertained to an accuracy of 0.05 gm.

The air leaving the second acid bottle is passed through a trap of cotton wool (Pl. XCIII) to catch any spray that might be carried from the sulphuric acid by the moving air. The quantity of acid that leaves the absorber is so small that the trap need not be weighed.

### SUPPLYING OXYGEN TO THE AIR

The oxygen supplied to the chamber to replace that absorbed by the active material is obtained from a cylinder which contains the gas under pressure. It is admitted at such a rate that the apparent volume of gas in the chamber as indicated by the tension equalizer is relatively constant. The regulation may be by hand; or the tension equalizer may be arranged to cause a valve in the oxygen feed line to be opened or closed as the volume of gas in the system diminishes or increases. The small cylinder with the gas-pressure-reducing valve attached weighs less than 10 kgm., and changes in the weight of it may be ascertained to an accuracy of 0.05 gm., which means that the actual volume of the gas admitted is known to within 50 c. c. With regularity in the rate of admission of oxygen, other methods of determining the quantity, as by means of an accurate meter carefully calibrated, or by the filling and emptying of a calibrated spirometer, are suitable. In the latter case a sensitive spirometer could serve also as a tension equalizer.

### CHANGES IN THE COMPOSITION OF THE RESIDUAL AIR

At the beginning and the end of each experimental period a portion of the air leaving the rotary pump is shunted through a train of small absorbing devices [1] and then through an accurate meter, which stands on the top shelf of the absorber table. The air leaving the meter is restored to that in the main line returning to the chamber. The weight of each small absorber, which is less than 100 gm., is ascertained to an accuracy of 0.1 mgm. The quantities of water vapor and carbon dioxid in the measured sample of air, as shown by the increase in the weights of the small absorbers, represent very accurately those of the atmosphere of the chamber. With such material as ripening fruit in the chamber, any change in the composition of the atmosphere occurs so slowly that it has no appreciable effect on the air of the chamber during the period of taking the sample. A fan to stir the air is unnecessary, the total volume being small when the quantity of active material used for experimental purposes is inclosed in the chamber. The air is withdrawn from the chamber through a pipe terminating at the floor in a cross with both ends open, while air is returned to the chamber through a pipe opening near the ceiling. The circulating air thus traverses the full depth of the chamber. At the usual rate of ventilation the total volume of air in the system completes the circulation several times per hour.

When a sample of air is desired for the determination of the proportion of oxygen present, it is usually taken from that returning from the absorbers to the chamber, no oxygen being admitted from the cylinder to the system at the time. Ordinarily this determination is not necessary, since by properly accounting for the different products removed from and admitted to the ventilating system, the quantity of oxygen consumed from the atmosphere may be computed.

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 311.

In the computation of the quantities of water vapor, carbon dioxid, and oxygen in the atmosphere of the chamber the actual volume of air in the chamber must be known, and this depends upon the capacity of the chamber under standard conditions of temperature (0°C.) and of barometric pressure (760 mm. of mercury) and the actual temperature and pressure of the air at the time the samples were taken. The barometric pressure of the air of the chamber, because of the tension equalizer, is always the same as that of the laboratory, which is determined to an accuracy of 0.01 mm. by means of a standardized barometer. The temperature of the air of the chamber is measured by means of an electric-resistance thermometer with the sensitive portion in the chamber and a temperature indicator outside. Either of two thermometers is available, one consisting of a single unit and the other of three units in series, which are modifications of the type of thermometer developed by Dickinson and Müller.[1] They are very sensitive and follow temperature changes rapidly. The single unit consists of a coil of nickel wire having a resistance of about 20 ohms at 20° C., wound on a very thin strip of mica, placed between two similar strips, and inclosed in a flat case of thin copper pressed firmly against the mica. The portion of the case which incloses the coil is about 15 cm. in length, 13 mm. in width, and less than 1.5 mm. in thickness. The case terminates at the top in a short tube, through which the leads are extended to the resistance wire, being sealed in the tube with a hard wax to exclude moisture from the interior of the case. Each of the three units in series is constructed like the one just described, except that it has only one-third the total amount of resistance wire; hence, the unit is shorter, the other dimensions being the same.

The leads from the resistance thermometer coils pass through the "outlet" mentioned on page 704 and extend to a multiple-switch (Pl. XCIV), by which either the single or the triple thermometer may be connected with the temperature indicator, which does not appear in any of the views shown. The latter device consists of a Wheatstone bridge having a slide wire by which the bridge circuit may be kept in balance with the thermometer coils at any temperature between 0° and 50° C. The readings of the bridge scale, when translated into temperature by means of a calibration curve, show changes to 0.1°. The effect of the resistance of the thermometer leads and of their change in resistance, due to change in temperature, is neutralized by compensating leads from the opposite side of the bridge, so that the measurements by means of the bridge are of a high order of accuracy; although, because of the small volume of air in the chamber, absolute accuracy of these determinations is of less significance than in experiments with the larger respiration apparatus.[2]

[1] Dickinson, H. C., and Mueller, E. F. New calorimetric resistance thermometers. *In* U. S. Dept. Com., Bur. Standards Bul., v. 9, no. 4, p. 483–492, 2 fig. 1913.

[2] Langworthy, C. F., and Milner, R. D. Op. cit., p. 312.

## DETERMINATION OF THE QUANTITY OF HEAT PRODUCED

The amount of heat resulting from the activity of the material in the respiration chamber is ascertained from determinations of (1) the quantity of latent heat in the water vapor of the outgoing water; (2) the quantity of sensible heat absorbed and carried away by water flowing in a coil of pipe in the chamber; and (3) the quantity of heat involved in changes in the temperature of the active material and of other objects in the chamber and also of the walls of the chamber. The gain or loss of sensible heat through the walls or in the ventilating current of air is prevented.

### LATENT HEAT

The quantity of water vapor carried from the chamber is determined from the gain in weight of the first sulphuric-acid bottle in the absorber train, as explained on page 706. If this quantity is multiplied by the factor 0.586, the product will be the number of Calories of latent heat at 20° C. carried from the chamber in the water vapor of the outgoing air.[1]

### SENSIBLE HEAT

Sensible heat emanating from the active material is removed from the chamber by a current of water flowing in a heat absorber, and the amount of heat thus removed in a given period is determined from the weight of water that flows through the absorber during the period and its temperature increase, with due allowance for the specific heat of the water at the mean temperature of the flow as compared with that at the temperature taken as standard. By controlling the rate at which water flows through the heat absorber, or the temperature at which it enters the absorber, the removal of heat is made to accord with its production, so that the temperature of the air of the chamber is kept as closely as possible to that which it is desired to maintain.

The heat absorber consists of 15 m. of copper tubing of 3-mm. bore in a double coil soldered to the upper and under surfaces of a piece of sheet metal 38 cm. square, with a double loop of pipe about 80 cm. long extending downward from each edge of the sheet. The absorber is removable and is slipped into position after the material under observation has been packed in the chamber. When in position, it is suspended with the sheet metal parallel to the ceiling of the chamber and about 25 mm. below it, with the double loops extending down the sides of the chamber and about 25 mm. from them.

### REGULATING THE RATE OF THE WATER FLOW

The water for the heat absorber flows from a constant level reservoir on a shelf above the calorimeter chamber, which is supplied from a tank on the lower shelf of the absorber table. The water that leaves the absorber is returned to the tank, from which it is raised again to the

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 315.

reservoir by a small gear pump driven by the motor that actuates the air pump. The overflow from the reservoir also returns to the tank. The same water is used continuously in this manner to eliminate the difficulty resulting when water directly from the city main is passed through the system, owing to the presence of air dissolved in the water. If the temperature of the water is raised, the air escapes and collects in bubbles in the pipe and forms temporary obstructions that cause irregularity in the rate of flow of water through the absorber. With well-filtered water in the system a rate of flow as low as 5 liters per hour has been maintained with such uniformity that it would be sufficient to collect the water leaving the heat absorbers at intervals instead of continuously. Slight changes in the rate may be effected by the adjustment of a glass rod, with a long tapering end which passes through a constricted orifice in one end of a glass T-tube in the water line.

That the air of the chamber may be kept at any desired temperature, water is usually allowed to flow through the heat absorber at a constant rate and the temperature of the ingoing water is varied in accordance with the quantity of heat to be absorbed. To bring this temperature under control, the water is first cooled below that at which it is to be used, and then heated to the desired temperature. In these circumstances regulation of temperature is accomplished simply by variation in the amount of heating, which is easily controlled automatically.

### REGULATING THE TEMPERATURE OF THE WATER FLOW

The water flowing from the reservoir to the heat absorber passes first through a pipe immersed in cold water to chill it, and then into a device called the preheater (Pl. XCIV) in which, by the conversion of electric current into heat in resistance coils inclosed in the water channel, the temperature of the water may be raised several degrees. The total heating effect of the device will increase the temperature of the water nearly 6° when the rate of flow is not over 500 c. c. per minute, and the heat may be added in small quantity. By this means the temperature of the water is raised near to that at which it is to enter the absorber. From this device, which is adjusted by hand, the water passes to the final heater (Pl. XCIV), which has a smaller capacity than the preheater, but is automatic and regulates the temperature within very narrow limits. The device is similar in some respects to that employed with the large calorimeter,[1] while in others it has been considerably simplified and improved.

The temperature of tne water is raised or lowered by increasing or decreasing the electric current flowing in a coil of resistance wire immersed in the water. This is accomplished by adjusting the position of the sliding contact on a rheostat wound with resistance wire of graduated

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 319.

cross section in series with the heating coil. The contact is moved by a motor-driven mechanism, the movement being governed by the deflection of the pointer of a sensitive galvanometer incorporated in the mechanism. The terminals of the galvanometer are connected with a special Wheatstone bridge (the temperature indicator shown at *F* in Pl. XCIV), one arm of which is a resistance thermometer installed in the upper half of the water channel in the final heater, so that it is submerged in the water flowing past the heating coil in the lower half of the channel. The slide wire of the bridge is calibrated to cover a range of temperature from 0° to 35° C., and the scale of the bridge is graduated to 0.1°. If the temperature of the stream of water in which the thermometer is immersed differs 0.05° from that at which the pointer of the temperature indicator is set, the needle of the galvanometer is deflected, the direction in which it swings depending upon whether the temperature of the water is too high or too low, and the amplitude of the swing depending upon the amount of the difference in temperature. The effect of any deflection is a shift in the position of the contact on the rheostat, which alters the current in the heating coil and thereby varies its heating effect. This continues until the water is brought to the desired temperature. The extent of change in the temperature of the water at any single shift of the contact on the rheostat varies according to the magnitude of the deflection of the pointer, from one of an extremely small fraction of a degree to one of about 0.1°. The cam shaft by which the contact is shifted rotates in less than 3 seconds, so that alteration in the heating current may occur every 3 seconds if necessary. Thus, the temperature of the water may be changed very quickly; or, in other words, any variation in its temperature from that desired may be rapidly corrected.

From the final heater the water flows to the bottom of a bottle of about 3 liters' capacity (Pl. XCIV, *B*), filled with small pieces of pumice, from the top of which it flows to the heat absorber at a very steady temperature.

It has been stated on page 710 that one purpose of controlling the temperature of the ingoing water is to keep the temperature of the air within the chamber as constant as possible. The operator counteracts any tendency towards change in the temperature of the air by changing the setting on the indicator for the temperature of the water entering the heat absorber. By a slight modification in arrangement this could be made automatic. The resistance thermometer for the temperature of the air in the chamber could be connected with the temperature indicator in place of the thermometer in the final heater, so that whenever the temperature of the air varied from that set on the indicator the device for regulating the temperature of the water entering the heat absorber would be changed in such a manner as to correct it.

MEASURING THE TEMPERATURE INCREASE

In the passage of the water through the heat absorber its temperature will increase according to its rate of flow and the quantity and activity of the material in the chamber. The accuracy with which the amount of heat carried from the chamber in the water current is measured depends upon that with which the temperature increase is determined. This is accomplished by means of electric-resistance thermometers and an automatic temperature recorder (Pl. XCV), in some respects similar to and in others different from that employed in connection with the large respiration calorimeter.[1]

In construction and characteristics the resistance thermometers are identical with those in the large calorimeter. They consist of two coils of platinum wire of equal resistance, which is about 25.5 ohms at 20° C., and have exactly the same coefficient of change in resistance with change in temperature, the resistance change of each being 0.1 ohm per degree. In each the resistance coil is encased in such a way that it is brought into intimate thermal contact with the flowing water and responds instantly and accurately to any change in its temperature. The water channels in which the resistance coils are installed are fitted into openings in the outlet described on page 704, to provide passage through the walls of the chamber for the ingoing and outgoing water, so that one coil acquires the temperature of the water just entering and the other that of the water just leaving the chamber.

The thermometers comprise two arms of a special Wheatstone bridge on opposite sides of a slide wire by which the bridge may be balanced for any inequality in the resistance of the two coils between 0.001 ohm and 0.2 ohm, resulting, respectively, from temperature differences of 0.01° and 2° between the ingoing and the outgoing water. The wire is calibrated so that temperature differences may be read directly from the scale. The total range of the instrument may be extended to indicate a difference as large as 5°. By means of resistance coils that may be connected in series with the slide wire as needed, the position of the balancing contact on the lower end of the wire may be made equivalent to a difference of 1°, 2°, or 3° between the thermometer coils, and the upper end 2° higher in each case.

The slide wire is incorporated in a mechanism which automatically balances the bridge for inequalities of resistance in the thermometers, and at the same time makes a graphic record of the balancing operations in terms of temperature difference and of time. The wire is mounted on the edge of a disk which is rotated to balance the bridge, while the balancing contact point remains fixed. The rotation of the disk, which is due to the action of one or the other of two cams on a shaft driven by

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 326.

a small electric motor, is governed by the deflection of the pointer of a very sensitive galvanometer, which is also incorporated in the mechanism, with its terminals connected with the Wheatstone bridge. When the bridge is in balance, the pointer remains at the zero position, and the slide wire is not moved; but any variation in the temperature of the water in either thermometer results in a change of resistance in the thermometer coils that upsets the balance of the bridge, the pointer swings to one side of the zero position or the other, according to the relation between the resistances of the opposite branches of the bridge, and the disk is turned in that direction in which the slide wire should be moved to restore the balance of the bridge. The amount of change in the position of the contact point on the slide wire is proportional to the magnitude of the swing of the pointer, which depends on the temperature difference in the thermometer coils. A difference of 0.005°, or even less, will upset the balance of the bridge sufficiently to cause a swing of the galvanometer pointer that will result in a movement of the disk. However large the temperature difference might be at any given instant, because of certain mechanical details connected with the mechanism for rotating the disk, stops are provided to limit the swing of the pointer either side of zero to that which would result from an inequality of resistance due to a difference of nearly 0.2° in the thermometer coils; but the cam shaft rotates every 5 seconds, and the disk may be moved that often if necessary; hence, the mechanism will keep the bridge balanced for inequalities resulting from any change of temperature difference in the ingoing and outgoing water up to 2° per minute.

The shaft on which the disk rotates also causes a pen to draw a line on ruled paper to show the direction and the distance that the slide wire had to be moved to balance the bridge. In the width of paper corresponding to the length of the slide wire that is equal to a difference of 2° there are 100 lines. The distance from line to line, which represents a temperature of 0.02°, is 2.5 mm. Hence, the temperature difference indicated by the position of the pen at any instant may be easily read to 0.01°.

The current in the bridge circuit is not sufficient to cause an increase in the temperature of the thermometers that will produce a movement of the pen even when the water is flowing through the thermometer at a rate much lower than the lowest that would be used with the apparatus.

A differential thermoelement is installed in the resistance thermometers so that the temperature difference of the water in the bulbs may be determined by means of a potentiometer as a check upon the measurement by the recorder. The Wheatstone bridge is provided with duplicate parts, which, by substitution, serve as means of checking the accuracy and constancy of the resistances of the bridge.

### CHANGE IN TEMPERATURE OF OBJECTS IN THE CHAMBER

Any change in the temperature of the walls of the chamber, or of the material confined within them, involves a quantity of heat for which allowance should be made in computing that produced in the chamber. For example, if the walls of the chamber are warmer at the end than at the beginning of the experiment, they have absorbed some of the heat that was produced in the chamber; while if they are cooler at the end of the experiment, some of their heat has been added to that in the chamber. The quantity of heat for which allowance must be made is computed from the change in the temperature of the walls and their hydrothermal equivalent—that is, the amount of heat involved per degree of temperature change in the walls.

The change in temperature is determined by electric-resistance thermometers devised for this apparatus. The resistance wire is wound in a flat coil about 5 cm. in diameter, which is firmly attached to one surface of a disk of stiff brass 55 mm. in diameter and 1.5 mm. thick. Through a hole in its center the disk is slipped over a short brass bolt projecting from the surface of the copper wall, so that by screwing a nut down on the bolt the disk may be clamped tightly against the wall, with the resistance wire between them. Between the wall and the wire are two or three layers of tinfoil to provide thermal contact in case of irregularity in the copper. The whole thermometer comprises 10 such coils, one for the top, one for the bottom, and one for the upper half and one for the lower half of each side. Each coil has a resistance of 45 ohms, but the 10 coils are connected in a series parallel arrangement to form a unit having a resistance of about 18 ohms at 20° C. The leads from this unit connect with the special switch and the Wheatstone bridge mentioned on page 709. The galvanometer will indicate a lack of balance due to a change of 0.05° in the temperature of the walls.

The most satisfactory data obtained in determining this factor indicate that for a change of 1° in the temperature of the walls the correction in the quantity of heat measured by the calorimeter would not exceed 1.5 Calories.

The correction involved in the change in temperature of the active material in the chamber is computed from the weight and specific heat of the material, and the temperature change as measured by an electric-resistance thermometer. One or the other of the two thermometers mentioned on page 709 is put into the mass of active material in such manner as to be in intimate contact with it. Tests with ripening fruit have shown that thermometers used in this manner indicate temperature change at least as accurately as a thermometer thrust into the flesh of one of the fruits.

PREVENTING GAIN OR LOSS OF HEAT IN THE CHAMBER

Part of the arrangement for preventing increase or decrease in the amount of the heat in the chamber by the passage of heat through the metal walls consists in duplicating the side walls, ceiling, and floor of the chamber by parallel surfaces of sheet metal attached to the outside of the wooden frame, as explained on page 705. For convenience, the metal walls which actually confine the chamber—in this connection all six surfaces being considered walls—are designated the inner walls, while the corresponding surfaces on the outside of the frame are called the outer walls. If the temperature of the outer wall is regulated so as to keep it always like that of the inner wall, neither will transmit excess of heat to the other, and consequently there will be no gain or loss of heat through the walls.

The temperature of the outer wall is regulated by heating and cooling the air in the narrow space between the wall and the heat-insulating cover described on page 705. The air is cooled by chilled water flowing in a small-bore copper tube in the space and it is heated by the conversion of electric energy into heat in a resistance wire parallel with the pipe. The wire and the pipe for controlling the temperature of the side walls are shown in Plate XCIII. The chilled water flows through the pipe continuously at such a rate that the air in the space will be too cool when the heating effect of the electric current in the resistance wire is near its minimum, and the current in the resistance wire is regulated until the air is heated to the desired temperature. In these circumstances the temperature of the air may be raised or lowered simply by varying the current in the resistance wire, which is accomplished by adjusting a rheostat in series with the wire.

The rheostat is adjusted automatically by a motor-driven mechanism (Pl. XCV). The resistances of the rheostat are arranged in a circle about a shaft by which the contact point is shifted to vary the amount of resistance in series with the heating wire. The direction in which the shaft will turn depends upon the deflection of the pointer of a galvanometer mounted in the shifting mechanism, with its terminals connected to a Wheatstone bridge, two arms of which consist of electric resistance thermometers attached to the inner and the outer metal walls of the chamber. The coils of these thermometers are identical in construction with those described on page 715 and are similarly attached to the outer surface of the inner wall and the inner surface of the outer wall, the disks on the inner wall forming one arm and those on the outer wall the opposite arm of the bridge. The two units are identical in resistance at the same temperature, and with the galvanometer employed they form a very sensitive differential thermometer that is influenced by small changes in the thermal condition of the walls. If the temperature of the outer wall differs by as much as 0.01° from that of the inner wall, the

resistances of the two parts of the thermometer will differ accordingly, and the pointer of the galvanometer will be deflected, the direction of deflection depending upon whether the outer wall is warmer or cooler than the inner, and the contact point of the rheostat will be shifted so as to increase or decrease the heating of the outer wall and bring it again into thermal equilibrium with the inner wall.

Thermal equilibrium is maintained in the walls by sections rather than as a whole. The resistance coils on the inner and outer metal walls are grouped so that the top, the sides, and the bottom of the chamber each has its own differential thermometer; and provision is likewise made for heating and cooling each section independently, so that thermal conditions in each one may be regulated regardless of those in the others. Furthermore, in order that there shall be no excess of sensible heat carried into or out of the chamber in the ventilating current of air, the temperature of the air entering the chamber is regulated to accord with that of the air leaving. The units of a differential resistance thermometer are inclosed in the pipes carrying the ingoing and outgoing air through the walls of the chamber. Just before the pipe for incoming air reaches the calorimeter a short section of it is inclosed in an electric heating device to warm the air, while inside the same section of pipe is a small copper tube conducting chilled water to cool the air. As in the control of the temperature of the walls, the water is kept running continuously and the temperature of the air is regulated by varying the electric current in the heater surrounding the air pipe. The four rheostats controlling the currents for heating the top, sides, and bottom outer walls of the chamber, and the ingoing air are adjusted by the same mechanism (Pl. XCV), which operates them successively, any changes that are needed in a given rheostat being made once every four minutes.

The widest difference between the respiration calorimeter described in the present article and the larger one previously described in this journal [1] is in the method of preventing gain or loss of heat in the chamber. The devices described in the paragraphs above render this apparatus quite largely automatic in its operations as a calorimeter, whereas the other calorimeter is controlled mainly by hand.

By means of a switch, also operated by the mechanism, the galvanometer which governs the action of the regulating mechanism upon the rheostats is connected successively across each of the four Wheatstone bridges of which the differential thermometers are integral parts, each pair of thermometers being combined with its own ratio coils to form a bridge. These four sets of coils are mounted in the same case (Pl. XCV) in such a manner that the permanence of resistance of each may be easily tested. The coils in each pair may be transposed by changing the position of two plugs, whereupon the galvanometer deflection will alter

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 326.

if the coils differ in resistance. Moreover, the ratio coils of one bridge may be combined with those of either of the other three to form a test bridge, all four arms of which should have the same resistance. With a very sensitive galvanometer across the bridge thus formed, any inequality in the coils would be detected. It is assumed that if there is no deflection the coils have not changed in resistance, since it is hardly probable that all the coils would have changed equally. If any change should be detected, by varying the combinations it would be possible to determine which pair of coils was at fault. By shifting the point of contact of the battery lead on a short wire joining the two coils, equality of resistance may be restored when the changes are slight. "No tests of this character have thus far indicated any need for change. Each bridge is also provided with a small variable shunt across a small resistance in series with one of the two differential thermometers to compensate for small inequalities in their resistances when at the same temperature.

## TESTS OF THE ACCURACY OF THE RESPIRATION CALORIMETER

The accuracy with which it is possible under given conditions to measure the factors studied by means of the respiration calorimeter is shown by a comparison of the determined amounts of oxygen consumed and of carbon dioxid, water vapor, and heat produced upon combustion of ethyl hydroxid in the chamber with those which should result from the combustion as calculated from the quantity of alcohol burned and the percentage of ethyl hydroxid in it.

A burner inside the chamber is connected with a small-bore copper tube that passes through the "outlet" in the wall of the chamber. To the exterior end of this tube is attached a glass U-tube with one long arm into which alcohol for the burner is fed by dropping from a supply bottle which may be weighed at intervals to determine the quantity burned. To test the apparatus under conditions equivalent to those of experiments in which it is used, the alcohol must be burned at a very slow rate. Some difficulties were experienced at first in attempts to burn as little as 1 gm. per hour with complete combustion of the alcohol at a constant rate and with inappreciable loss by evaporation from the long arm of the U-tube. These were due in part to the fact that the opening in the "outlet" through which the alcohol tube passed is considerably above the level at which it is desired to have the combustion take place in the chamber. As a result of this condition, in all the tests thus far made the level at which the alcohol was maintained in the vertical tube was above that at which it was burned, attempts to feed the burner by siphon having proved unsuccessful. It was necessary to devise a burner which would overcome the effect of the pressure of the alcohol in the feed tube upon the rate of flow.

Burners of small-bore glass tubing of various diameters and with wicks of cotton, of glass wool, and of ignited asbestos, packed so as to allow

the alcohol to escape at the desired rate, were tried, but most of them were worthless because after combustion had continued a short time the flow of alcohol would begin to diminish and finally would be stopped entirely by material deposited in the top of the wick. This would occur even when the upper part of the wick was removed so that there was clear alcohol to a depth of 3 mm. or more below the flame. The phenomenon appeared to be associated with incomplete combustion of the alcohol, because whenever it occurred evidence that combustion was not complete could be found in the air of the chamber. That the material deposited in the wick was not in solution or in suspension in the alcohol was indicated by the fact that a sample of 100 gm. from the supply bottle when evaporated left a residue less than 0.1 mgm.

Some successful results were obtained with a burner of very thick wall and a bore of approximately 1 mm., with glass wool for a wick. When the glass wool was sufficiently tamped some pressure was necessary to force alcohol through it at the desired rate. The chief objection to this burner was the tendency of the thick tube to crack when the alcohol was lighted. Alcohol was fed from the supply bottle by dropping at such a rate that the level of the alcohol would remain at a mark on the long arm of the U-tube indicating the height which had been found by trial to produce sufficient pressure to keep the alcohol burning at the desired rate. This U-tube was of small bore to reduce the surface from which evaporation could take place, and the open end of the tube was nearly closed by the constricted nozzle of the tube from the supply bottle, leaving only a small space through which vapor could escape.

The results obtained with a burner consisting of two concentric small tubes and a wick of asbestos tape filling the narrow annular space between them were also quite satisfactory. No products of incomplete combustion were found in the air of the chamber when alcohol was burned in either of these burners at a rate even lower than 1 gm. per hour. The data in Table I show the results of two representative tests.

TABLE I.—*Data obtained in the combustion of alcohol in the respiration calorimeter*

| Date. | Duration. | Weight of alcohol burned. | Water. | | Carbon dioxid. | | Oxygen. | | Heat. | | Respiratory quotient, $CO_2:O_2$. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Found. | Required. | Found. | Required. | Found. | Required. | Found. | Required. | |
| 1914. | *Hours.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Cal.* | *Cal.* | |
| Jan. 21..... | 5 | 9.20 | 9.7 | 10.7 | 15.7 | 16.2 | 17.4 | 17.7 | 61.2 | 60.4 | 0.655 |
| | 5 | 9.22 | 10.1 | 10.7 | 16.0 | 16.2 | 17.3 | 17.7 | 61.0 | 60.5 | .673 |
| | 10 | 18.42 | 19.8 | 21.4 | 31.7 | 32.4 | 34.7 | 35.4 | 122.2 | 120.9 | .663 |
| Jan. 27..... | 11.75 | 10.34 | 11.8 | 11.9 | 16.7 | 17.2 | 18.4 | 18.7 | ..... | ..... | .660 |

In the test of January 21 the alcohol was burned at a rate averaging slightly more than 1.8 gm. per hour. Almost exactly the same total quantity was burned in each of the two consecutive 5-hour periods com-

prising the test, and the determinations of carbon dioxid, oxygen, and heat in one period agree quite closely with those in the other, while the discrepancies between the quantities found and those required are for the most part small. The ratio of the volume of carbon dioxid produced to that of oxygen consumed in the test was 0.663, whereas the theoretical respiratory quotient for the combustion of alcohol is 0.667.

The only test in which less than 1 gm. of alcohol per hour was burned for any considerable period was that on January 27, which continued nearly 12 hours, at a rate of combustion averaging only 0.88 gm. per hour. There was a close agreement between the quantities computed and those determined in the measurement of gaseous exchange in this test also, but the heat production was not determined. These results are quite typical of all those obtained in tests of this character. In none of them have there been wider discrepancies than these shown between the measured and calculated quantities, the reasons for which were not ascertained and which could not have been avoided.

The accuracy with which heat generated in the calorimeter chamber can be determined is tested also by converting a known amount of electrical energy into heat in a resistance coil suspended in the chamber and measuring the heat with the calorimeter. In a test made on February 3, 1914, a current of 0.087 ampere was passed through a resistance coil of 1,680 ohms at an average pressure of 146.5 volts, generating

10.97 Calories of heat per hour according to the formula $\frac{I^2R}{4.183}$ = small

Calories per second at 20° C. The quantity of heat measured by the calorimeter was 11.04 Calories in the first hour and 11.08 Calories in the second hour of the 2-hour test. During the second hour the increase in the temperature of the water that flowed through the heat absorber in the chamber was measured by a potentiometer and the differential thermoelement installed in the resistance thermometers (see p. 713) as a check on the measurement by the thermometers themselves. The average temperature difference was 1.42° as indicated by the resistance thermometers and recorder and 1.40° by the thermoelement and potentiometer. The discrepancy between the computed and the measured amounts of heat in the second period of this test is wider than that in any other electric test with this respiration calorimeter. The closest agreement was that obtained in a test which continued only 1 hour, on November 2, 1912, in which the amount of heat computed to have been generated in the chamber was 7.54 Calories and that measured by the calorimeter was 7.56 Calories.

Both the electric and the alcohol tests indicate that measurements can be made with this respiration calorimeter to a high degree of accuracy.

## PLATE XCII

### General view of the respiration calorimeter

*A*, Chamber inclosed in heat-insulating cover. *B*, Tension equalizer to maintain atmospheric pressure in the air of the chamber. *C*, Absorber table. *D*, Rotary pump to maintain air circulation. *E*, Motor to drive pump. *F*, Bottles containing sulphuric acid to remove water vapor from circulating air. *G*, Large U-tube, containing soda-lime to remove carbon dioxid from the air. *H*, Bottle containing sulphuric acid to catch the water vapor from the soda-lime. *I*, Bottle containing cotton to catch sulphuric acid vapor. *J*, Small absorbers for determining water vapor and carbon dioxid in residual air. *K*, Meter to measure the sample of residual air. *L*, Reservoir to maintain a constant pressure of water in the heat absorber in the chamber. *M*, Tank to catch water flowing from the heat absorber. *N*, Pump to raise water from the tank to the reservoir. *O*, Devices for automatically controlling and recording temperatures.

L
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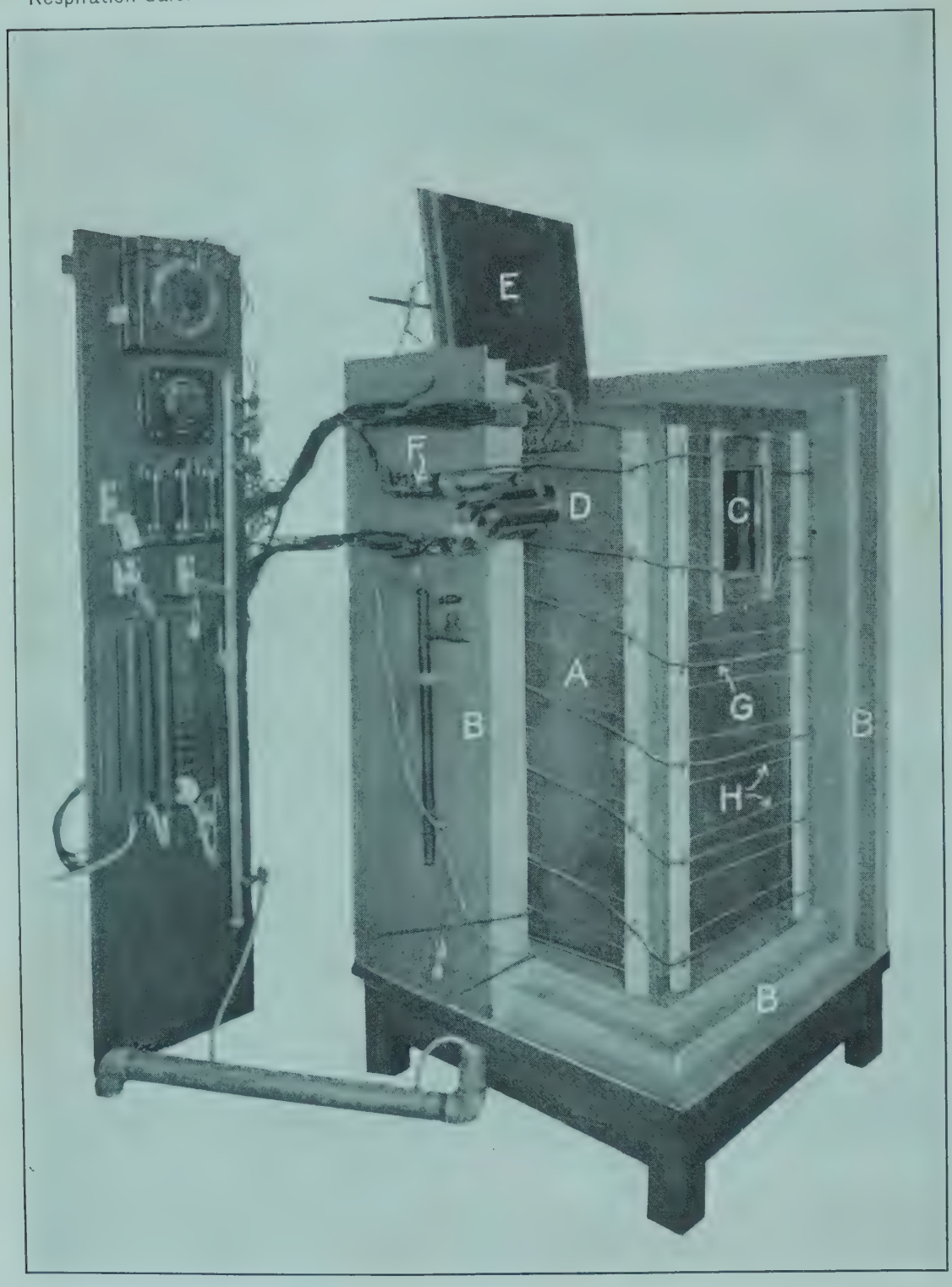
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B

## PLATE XCIII

Chamber with part of outer covering removed

*A*, Double metal wall chamber. *B*, Heat-insulating outer cover. *C*, Window to chamber. *D*, Outlet providing passage for pipes, wires, etc., through the walls of the chamber. The exterior ends of the resistance thermometers for ingoing and outgoing water are seen projecting from the outlet. *E*, Removable top of chamber. *F*, Device for heating the air entering the respiration chamber. *G*, Small pipe carrying water for cooling the outer metal wall of the chamber. *H*, Electric-resistance wire carrying current for heating the outer wall.

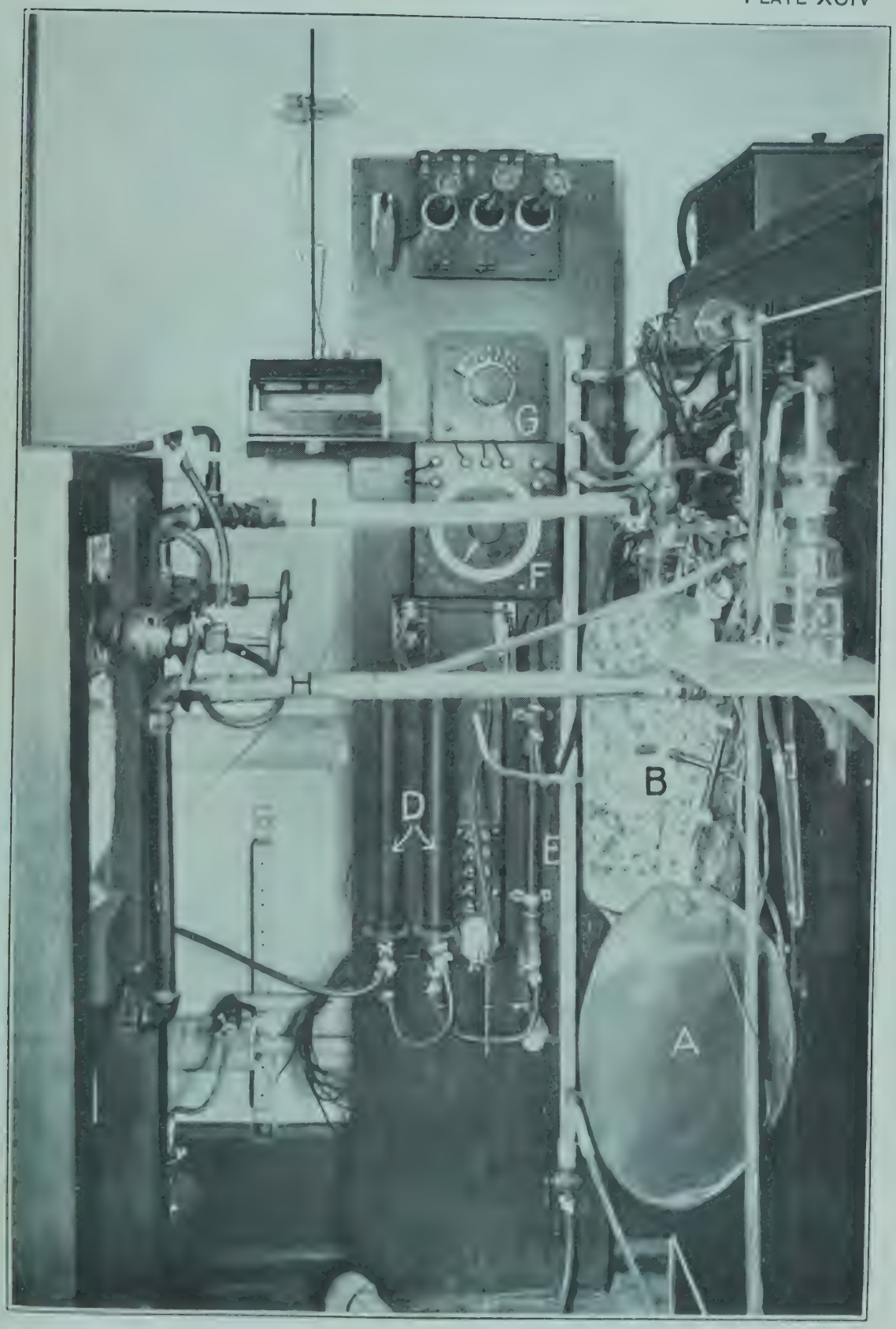
## PLATE XCIV

### Apparatus connected with the respiration calorimeter

*A*, Tension equalizer. *B*, Mixing bottle for equalizing the temperature of water entering the heat absorber. *C*, Device for heating air entering the respiration chamber. *D*, Preheater, and *E*, final heater, for raising the temperature of water entering the heat absorbers. There is an electric-heating coil in the lower half and an electric-resistance thermometer in the upper half of the final heater. *F*, Temperature indicator comprising part of the apparatus for controlling the temperature of the water entering the heat absorber. This device is connected with the resistance thermometer in the final heater and with the galvanometer in the controlling mechanism marked *B* in Plate XCV. *G*, Multiple-point switch for connecting the resistance thermometers for the metal walls and air of the chamber with the Wheatstone bridge for measuring their temperatures. *H*, Tube conducting air from the respiration chamber to the rotary air pump. *I*, Tube conducting air from the purifying devices to the respiration chamber.

Respiration Calorimeter

PLATE XCIV



Respiration Calorimeter

## PLATE XCV

Devices for controlling and recording temperatures

*A*, Mechanism for shifting the contact on the rheostats controlling the current for heating the outer walls of the calorimeter chamber and the ingoing air. The rheostats are almost entirely hidden at the rear of the case inclosing the mechanism. *B*, Ratio coils for the four bridges governing the action of the shifting mechanism *A* are combined in this box, together with means for checking the constancy of the resistance of the coils and for correcting slight inequalities in them and also to compensate for small differences in the pair of resistance thermometers forming the other arms of each bridge. *C*, Mechanism for shifting the contact on the rheostat controlling the current in the heating coil in the final heater, shown at *E* in Plate XCIV. The rheostat is below the case inclosing the shifting mechanism. *D*, Temperature-difference recorder (self-balancing Wheatstone bridge) for continuously recording the difference between the temperature of the water entering and that leaving the heat absorber. *E*, "Check box" containing the ratio coils of the bridge for temperature difference measurements and coils for extending the range of differences measured, with means for checking the constancy of the resistances of the coils and the accuracy of the recorder readings and also for compensating for slight differences in the resistance of the thermometer coils when they are at the same temperature.

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# MOTTLE-LEAF OF CITRUS TREES IN RELATION TO SOIL CONDITIONS

By LYMAN J. BRIGGS, *Biophysicist in Charge*, C. A. JENSEN, *Assistant in Plant Malnutrition*, and J. W. MCLANE, *Laboratory Assistant, Biophysical Investigations, Bureau of Plant Industry*[1]

## INTRODUCTION

"Mottle-leaf" is a term applied in California to a mottled or spotted condition of the leaves of Citrus trees (Pl. XCVI). The affected portions of the leaf appear to be nearly or quite devoid of chlorophyll and are light yellow in color. In the first stages of the disease irregular spots several millimeters in diameter appear between the larger veins, usually midway between the midrib and the margin. The half of the leaf next to the tip is often first affected. In the more advanced stages, the spots are larger and more numerous, until finally the only chlorophyll remaining is confined to the midrib and the larger veins. The various stages are illustrated in Plate H. The condition is distinguished from what is generally termed "chlorosis" by the fact that the areas surrounding the yellowish spots retain their normal green color, at least until the spots embrace a large proportion of the leaf. The term "mottle-leaf" as here used is also to be understood as not including that type of functional disturbance sometimes found in Citrus leaves in which the midrib and veins are lighter in color than the surrounding tissue.

Mottle-leaf in its advanced stages is accompanied by a serious reduction in the yield and in the size and quality of the fruit. The foliage becomes thin and weak, with many very small leaves (Pl. XCVII); and the ends of the branches have a brushy appearance, owing to the development of numerous small weak twigs.

## DISTRIBUTION OF MOTTLE-LEAF

Mottle-leaf is at present quite widely distributed through the Citrus areas of California, but is, as a whole, worse in the southern sections of the

[1] The writers are indebted to the University of California Citrus Experiment Station and Graduate School of Tropical Agriculture at Riverside for many courtesies and facilities extended during the course of this work, and to Dr. H. L. Shantz and Mr. R. L. Piemeisel, of the Office of Alkali and Drought Resistant Plant Investigations, for their cooperation in the work preliminary to this investigation.

The writers also wish to express their obligation to the Citrus growers of the sections studied for their cooperation in supplying information regarding the field treatments of their groves and assistance in other ways.

State. It is not a new trouble in California, having been observed by some of the growers at least 15 years ago. During the last three or four years the mottling has become much more pronounced in the groves first affected. Other groves which are reported to have been relatively free from the trouble a few years ago are now badly mottled. There are many groves in the affected districts, however, that show little or no mottling at present.

## FACTORS SUGGESTED AS CAUSAL AGENTS IN MOTTLING

The cause of mottling is a much disputed point. Various factors have been assigned as causal agents, such as an excess of lime, magnesium, or organic matter; a deficiency of lime, iron, or organic matter; frost; poor drainage, etc. Smith and Smith [1] conclude from their observations that the most prevalent and typical form of mottle-leaf is due to an irregular supply of moisture and plant food. No fungus or bacterium has yet been proved to be causally associated with mottle-leaf. Thomas,[2] however, has shown that the Citrus-root nematode (*Tylenchulus semipenetrans* Cobb) is widely distributed in districts in which mottle-leaf occurs, but is not invariably found on the roots of affected trees, and the extent to which mottling can be directly induced by such parasitism has not yet been determined.

One of the most striking features of mottle-leaf is the fact that the deficiency in chlorophyll is first in evidence in those portions of the leaf farthest removed from the midrib and largest veins; in other words, farthest from the main conducting channels of the leaf (Pl. H). This suggests a deficiency in the available supply of some substance essential in the formation of chlorophyll. The entire supply of this substance is apparently used by those portions of the leaf near the conducting channels, the supply being insufficient to reach the more remote portions of the leaf. That the disappearance of the chlorophyll is due to the absence of some essential constituent in the leaf rather than to the presence of some deleterious substance is also indicated by the fact that the chlorophyll next to the midrib and larger veins is the last to disappear. If the plant were absorbing something which reacted unfavorably on the chlorophyll, the effect of such absorption might be expected to be first in evidence nearest the veins. This analysis of the problem is to be considered simply as a working hypothesis which up to the present appears to accord with the observations.

The marked reduction in the yield of marketable fruit from badly mottled trees and the decrease in vigor led to the undertaking of a systematic survey of a number of groves in districts in which mottle-leaf

[1] Smith, R. E., and Smith, Elizabeth H. California plant diseases. Cal. Agr. Exp. Sta., Bul. 218, p. 1139. 1911.

[2] Thomas, E. E. A preliminary report of a nematode observed on citrus roots and its possible relation with the mottled appearance of citrus trees. Cal. Agr. Exp. Sta., Cir. 85, 14 p., 8 fig. 1913.

occurs with a view to determining the extent of its correlation with soil conditions. The results of these investigations form the subject of this paper.

## FIELD SURVEY

As a basis for the investigation a field survey was made of about 175 orange and lemon groves near Riverside, Redlands, Highland, and Rialto, Cal.; a few groves were also examined in the Ontario, Pomona, and Azusa districts.

Ten or twelve representative trees were selected from each grove examined, usually a 10-acre block. The percentage of mottled leaves on each tree was determined by two men working independently. Soil samples were also taken near the same trees in 1-foot sections to a depth of 3 feet, the samples for a given foot section being combined. The fertilizer treatment of each grove and the cultivation and irrigation methods employed were also ascertained as accurately as the records of each grower would permit.

The soil samples representing each grove were promptly air-dried, and the organic carbon, "humus," total nitrogen, mineral carbonates, and bicarbonates determined in each sample. The moisture equivalent of each sample was also determined in order to compare the moisture retentiveness of the soils.

The limiting of the sampling to a depth of 3 feet was based upon the results of numerous observations, which showed that the feeding roots of orange and lemon trees do not as a rule extend much beyond this depth. The taproots, of course, go deeper when the soil conditions permit; but the feeding root system spreads out laterally near the surface, and this lateral feeding system does not ordinarily fully occupy the ground even to a depth of 3 feet. Excavations in the districts examined showed repeatedly that the main feeding root system was found usually from within a few inches of the surface to a depth of 18 to 24 inches.[1] Soil-moisture determinations in orange groves, to be presented in another paper, also showed that when the upper 3-foot layer dried out below the wilting coefficient, the tree could not get enough water to keep from wilting, even with available moisture immediately below this layer.

## EXTRANEOUS FACTORS COMPLICATING THE CORRELATION OF MOTTLING WITH SOIL CONDITIONS

The study of the correlation between the degree of mottling and the general soil conditions of the groves is complicated by a number of extraneous factors. One of these is the kind of stock on which the selected buds are grafted. Other conditions being the same, a tree top on sour-orange stock is likely to show more mottling than one on sweet-

[1] Striking exceptions are, however, occasionally met with. Dr. H. J. Webber, Director of the University of California Citrus Experiment Station, informs the writers that in the Claremont section he has observed fine fibrous roots at a depth of 14 feet.

orange stock. In some instances also, tops on grapefruit stock were found to be more mottled than tops on sweet-orange seedlings. One specific instance of two adjoining navel-orange groves under the same ownership illustrates the case. One grove is on lemon stock, the other on sweet-orange stock. The trees on lemon stock showed 70 per cent of their leaves mottled, and those on the sweet-orange stock 50 per cent. Also, instances were found where individual orange trees on lemon stock were much more mottled than the surrounding trees on sweet-orange stock. As it is frequently impossible to obtain definite information about the stock used, this factor complicates the investigation. The physiological behavior of the buds on various stocks would be an interesting study in this connection.

Frosts and severe winds tend to increase mottling. After a strong, dry, north wind late in the fall of 1912 the leaves on the north side of the trees were more mottled than those on the opposite side. The new leaves put out later on the north side of the tree, however, were less mottled; and during the summer of 1914, the period covered by the field survey, the leaves on the south side of the trees were generally more mottled than those on the north side. Even in very severe cases of mottling, large healthy leaves are also often found in the center of the trees. This suggests that strong sunlight may increase mottling; and if so, the effectiveness of this agency would vary with the size of the trees and the closeness of planting.

Badly mottled trees cut back and rebudded on the stumps produce badly mottled new top growth, and the mottling persists unless the soil treatment is changed.

## FERTILIZERS IN RELATION TO MOTTLING

The results of the field survey showed that groves which were plentifully supplied with organic material, either in the form of manures or green cover crops, were less mottled than those that had been fertilized entirely with commercial fertilizers. Several growers stated that they had cured mottle-leaf in limited areas by a liberal application of barnyard manure. It was also found in the case of all the groves included in this field study that each grove that had been fertilized with commercial fertilizers alone and kept under clean cultivation was badly mottled. This condition was especially marked in groves in which sodium nitrate had been employed for a number of years as the principal or only fertilizer. On the other hand, some groves that had received organic fertilizer were also badly mottled. This latter fact has discouraged many growers from using such material, especially manures, which usually have to be purchased and shipped in at high cost.

It was also observed that plowsole (an incipient hardpan just below the cultivated layer) frequently accompanied a badly mottled condition of the tree. Numerous observations have shown that the plowsole is a serious

obstacle in irrigation and gives rise to a droughty condition in the areas affected. The association of mottling with an inadequate soil-moisture supply appears in some instances to be clearly indicated. The relationship, so far as plowsole is concerned, is, however, complicated by the fact that plowsole is often, though not necessarily, associated with a low humus content.

### RELATION OF MOTTLING TO YIELD

Badly mottled trees produce smaller fruits and a smaller number of fruits per tree than trees not mottled, and severely mottled branches produce less fruit buds. A slight mottling of the leaves does not appear to have any serious effect on the yield of fruit. The results of the field observations indicate that if less than 20 per cent of the leaves show mottling, the yield is not measurably decreased. The yields of oranges and lemons in the groves studied were obtained in most cases as far back as 1907, but the freezes of 1912 and 1913 proved such a disturbing factor both as regards yield and tree condition that it was not found possible to establish any relation between the yield and the mottling as determined in 1914.

### SUSCEPTIBILITY OF DIFFERENT CITRUS TREES TO LEAF MOTTLING

Mixed groves of lemons and oranges were not found in the field survey, so that the relative mottling of the two species under the same cultural conditions could not be directly determined. From indirect comparison there appears to be no great difference in this respect. Grapefruit and tangerine mottle readily, but no opportunity was presented for a direct comparison with lemon or orange trees. There are few tangerines produced in the areas studied.

There seemed to be no difference between the Washington Navel and the Thompson Improved Navel so far as susceptibility to mottling was concerned. Where differences in mottling were found, it was also found that the two varieties were on different stocks. One mixed grove of Washington Navels and Valencias was studied, in which the two varieties were alternated in the same row, so that the conditions were the same for each. In this case both varieties were equally mottled.

## RESULTS OF THE SOIL ANALYSES

### METHODS OF SOIL ANALYSIS EMPLOYED

The total carbon was determined by boiling 20 gm. of soil with 50 or 75 c. c. of a mixture of sulphuric acid and potassium bichromate, using the larger amount with soils containing more than the average amount of organic matter. The acid mixture was made up in the proportion of 120 gm. of the bichromate to 1,000 c. c. of concentrated sulphuric acid. The carbon dioxid was absorbed in $N^2/_3$ sodium hydrate in a bead tower and the whole of the hydrate solution removed and titrated.

The inorganic carbon (from mineral carbonates) was determined by boiling 20 gm. of soil with 50 c. c. of normal phosphoric acid under a partial vacuum of about 68 cm. of mercury, absorbing the carbon dioxid in *N*⅔ sodium hydrate and titrating as in the case of total carbon. The phosphoric acid liberates the carbon dioxid in mineral carbonates or bicarbonates, but does not appear to attack appreciably the organic matter.

The humus was determined by removing the calcium from 10 gm. of soil with dilute hydrochloric acid (1 per cent), washing out the chlorids, extracting the soil with 500 c. c. of 4 per cent ammonia for 24 hours, and measuring the intensity of the humus color in a colorimeter against a standard humus solution.

The percentage of soluble bicarbonates was determined by shaking the soil with distilled water and allowing it to stand overnight. The clear supernatant liquid was pipetted off the following morning into a Jena flask, a few drops of phenolphthalein added, the flask covered with a watch glass and the solution boiled on a hot plate; while boiling, the red color was titrated out with *N*/10 hydrochloric acid.

The total nitrogen was determined by the modified Kjeldahl method, which includes the nitrogen of nitrates.

## ORANGE SOILS

The difficulties encountered in correlating tree growth with soil conditions as determined by a laboratory examination are generally recognized. The soil environment of a tree is by no means uniform, and a soil sample at best represents only the average soil condition, and wholly disregards the local variations in root distribution. In the present investigation the correlation between mottling and soil composition is further complicated by the fact that an orange or lemon tree is, generally speaking, slow in response to fertilizer stimuli under the method of orchard management prevailing in the area studied. An application of barnyard manure, for example, even when thoroughly worked into the soil, does not cause tree response until some time after the manure has decomposed. Under such circumstances a soil sample may not represent the soil conditions responsible for the condition of the trees at the time of sampling

TABLE I.—*Analyses of orange-grove soils in California to a depth of 3 feet*

| No. | Percentage of— | | | | | Ratio of— | | | | Moisture equivalent. | Trees. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Humus. | Total nitrogen. | Organic carbon. | Carbonates. | Bicarbonates. | Humus to lime. | Carbon to humus. | Nitrogen to humus. | Nitrogen to carbon. | | Age. | Percentage of mottled leaves. | Variety. | Location. |
| | | | | | | | | | | | *Years.* | | | |
| 1 | 0.033 | 0 | 0.271 | 0.051 | 0.023 | 0.66 | 8.15 | 0 | 0 | 10.4 | 21 | 89 | Valencia | A. H. 74-2. |
| 2 | .033 | .034 | .267 | .051 | .023 | .66 | 8.00 | 1.01 | .13 | 10.4 | 21 | 73 | do | A. H. 74-3. |
| 3 | .041 | .037 | .244 | .062 | .021 | .67 | 5.90 | .88 | .15 | 13.6 | 21 | 88 | do | A. H. 52-4, exp. 1. |
| 4 | .042 | .034 | .247 | .065 | .028 | .63 | 5.95 | .82 | .14 | 13.2 | 21 | 90 | do | A. H. 52-4, exp. 2. |
| 5 | .055 | .038 | .255 | .075 | .028 | .73 | 4.65 | .70 | .15 | 14.1 | 21 | 93 | do | A. H. 52-4, exp. 3. |
| 7 | .047 | .026 | .126 | .030 | .017 | 1.58 | 2.65 | .55 | .21 | ..... | 16 | 58 | Washington | Hungate. |
| 8 | .049 | .037 | .256 | .071 | .025 | .69 | 5.08 | .75 | .14 | 13.6 | 21 | 90 | Valencia | A. H. 52-4, exp. 4. |
| 10 | .059 | .038 | .276 | .082 | .026 | .72 | 4.70 | .64 | .14 | 13.6 | 21 | 90 | do | A. H. 52-4, exp. 5. |
| 11 | .057 | .034 | .189 | .015 | .023 | 3.79 | 3.33 | .60 | .18 | 10.7 | ..... | 76 | do | N. O. Co. Palmyr. |
| 12 | .069 | .032 | .272 | .034 | .021 | 2.02 | 3.98 | .46 | .12 | 12.6 | 21 | 93 | Washington | A. H. 52-2. |
| 13 | .067 | .045 | .251 | .041 | .018 | 1.37 | 3.76 | .68 | .18 | 14.9 | 13 | 2 | do | N. O. Co. Vict. 10 |
| 14 | .076 | .056 | .229 | .054 | .028 | 1.41 | 3.00 | .74 | .25 | 11.2 | 21 | 91 | Valencia | A. H. 63-3. |
| 15 | .078 | .058 | .262 | .417 | .024 | .19 | 3.38 | .74 | .22 | 14.7 | 15 | 9 | Thompson | N. O. Co. M. A. C. |
| 16 | .082 | .033 | .249 | .018 | .022 | 4.45 | 3.05 | .41 | .13 | 9.4 | ..... | 25 | Washington | L. V. W. B., Oat. 5. |
| 17 | .084 | .036 | .213 | .026 | .030 | 3.23 | 2.54 | .43 | .17 | 12.0 | ..... | 33 | do | L. V. W. B., Oat. 9. |
| 18 | .085 | .034 | .192 | .053 | .017 | 1.58 | 2.32 | .41 | .18 | 14.3 | 14 | 4 | do | N. O. Co. Vict. Hill. |
| 19 | .086 | .040 | .192 | .044 | .022 | 1.95 | 2.22 | .47 | .21 | 10.6 | 14 | 30 | Thompson | N. O. Co. Crouch. |
| 20 | .095 | .036 | .294 | .018 | .022 | 5.15 | 3.10 | .38 | .12 | 10.5 | 21 | 66 | Washington | A. H. 62-1. |
| 21 | .091 | .028 | .183 | .018 | .023 | 5.20 | 2.00 | .30 | .15 | 10.2 | ..... | 68 | do | L. V. W. B. Palmyr. |
| 22 | .093 | .032 | .195 | .012 | .022 | 7.95 | 2.10 | .35 | .17 | 12.1 | ..... | 70 | Thompson | L. V. W. B. Low. Cer. |
| 23 | .099 | .029 | .199 | .012 | .026 | 8.45 | 2.01 | .30 | .15 | 11.5 | ..... | 55 | do | L. V. W. B. Palmyr. |
| 24 | .091 | .052 | .355 | .052 | .014 | 1.76 | 3.91 | .57 | .15 | 11.1 | 23 | 73 | Washington | N. O. Co. Shoem'k'r. |
| 25 | .093 | .041 | .236 | .027 | .023 | 3.48 | 5.53 | .45 | .18 | 16.4 | 12 | 12 | do | N. O. Co. Vict. 20, exp. 10. |
| 26 | .100 | .037 | .263 | .114 | .043 | .87 | 2.64 | .37 | .14 | 16.2 | ..... | 50 | do | Elsinore Rd. |
| 27 | .104 | .038 | .250 | .073 | .026 | 1.43 | 2.40 | .36 | .15 | 12.5 | 21 | 71 | Valencia | A. H. 80-4. |
| 28 | .106 | .030 | .236 | .023 | .021 | 4.75 | 2.21 | .29 | .13 | 9.8 | 21 | 85 | do | A. H. 80-1. |
| 29 | .105 | .052 | .249 | .055 | .025 | 1.92 | 2.37 | .50 | .21 | 11.2 | 15 | 39 | do | N. O. Co. Viv. |
| 30 | .105 | .044 | .228 | .099 | .023 | 1.06 | 2.18 | .42 | .19 | 16.3 | 13 | 21 | Washington | N. O. Co. Vict. 20, exp. 9. |
| 31 | .108 | .036 | .184 | .149 | .020 | .73 | 1.70 | .34 | .20 | 16.8 | 12 | 8 | do | N. O. Co. Eur. 10. |
| 32 | .104 | .029 | .185 | .018 | .027 | 5.68 | 1.77 | .28 | .16 | 11.5 | ..... | 82 | W. and V | Viv. |
| 33 | .110 | .039 | .203 | .010 | .023 | 10.90 | 1.83 | .35 | .19 | 11.8 | ..... | 81 | Valencia | L. V. W. B. Sun. Mt. |
| 34 | .110 | .031 | .201 | .013 | .018 | 8.20 | 1.83 | .28 | .16 | 10.8 | ..... | 37 | do | L. V. W. B. Sun. Mt. |
| 35 | .117 | .034 | .230 | .019 | .020 | 5.13 | 1.95 | .29 | .15 | 9.7 | 21 | 56 | Washington | A. H. 61-1. |
| 36 | .113 | .034 | .312 | .025 | .019 | 4.50 | 2.76 | .30 | .11 | 12.1 | ..... | 22 | do | L. V. W. B., Oat. 8. |
| 37 | .104 | .031 | .226 | .023 | .026 | 4.44 | 2.18 | .30 | .14 | 12.4 | ..... | 50 | do | L. V. W. B., Up. Cer. |
| 38 | .115 | .043 | .282 | .018 | .024 | 6.30 | 2.45 | .37 | .15 | 12.0 | ..... | 52 | Washington | Hae. Sun. Mt. |
| 39 | .114 | .053 | .242 | .040 | .018 | 2.86 | 2.12 | .46 | .22 | 16.9 | 13 | 6 | do | N. O. Co. Vict. 13. |
| 40 | .116 | .044 | .262 | .025 | .018 | 4.65 | 2.24 | .38 | .17 | 16.8 | 13 | 2 | Valencia | N. O. Co. Vict. 8. |
| 41 | .119 | .034 | .280 | .084 | .017 | 1.41 | 2.36 | .37 | .12 | 16.4 | 13 | Trace. | Washington | N. O. Co. Vict. 17. |

TABLE I.—*Analyses of orange-grove soils in California to a depth of 3 feet*—Continued

| No. | Percentage of— | | | | | Ratio of— | | | | Moisture equivalent. | Trees. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Humus. | Total nitrogen. | Organic carbon. | Carbonates. | Bicarbonates. | Humus to lime. | Carbon to humus. | Nitrogen to humus. | Nitrogen to carbon. | | Age. | Percentage of mottled leaves. | Variety. | Location. |
| | | | | | | | | | | | *Years.* | | | |
| 42 | 0.118 | 0.029 | 0.218 | 0.017 | 0.021 | 7.10 | 1.84 | 0.24 | 0.13 | 13.9 | 12 | Trace. | Washington | N. O. Co. Eur. 7. |
| 43 | .122 | .034 | .201 | .028 | .033 | 4.46 | 1.63 | .28 | .17 | 11.5 | 13 | 18 | Thompson | N. O. Co. Pach. 4. |
| 44 | .128 | .038 | .253 | .039 | .024 | 3.27 | 1.98 | .30 | .15 | 18.2 | ..... | 57 | Washington | Colton Ave. Sm. |
| 45 | .097 | .036 | .270 | .068 | .030 | 1.42 | 2.38 | .37 | .14 | 15.5 | ..... | 55 | .....do | Colton Ave. Sm. |
| 46 | .132 | .038 | .267 | .073 | .025 | 1.82 | 2.02 | .28 | .14 | 16.3 | 13 | 32 | .....do | N. O. Co. Vict. 12, exp. 3. |
| 47 | .138 | .046 | .238 | .104 | .024 | 1.32 | 1.73 | .34 | .19 | 16.3 | 13 | 46 | .....do | N. O. Co. Vict. 12, exp. 5. |
| 48 | .134 | .039 | .212 | .098 | .020 | 1.34 | 1.58 | .29 | .19 | 16.5 | 13 | 11 | .....do | N. O. Co. Vict. 12, exp. 6. |
| 49 | .131 | .039 | .230 | .033 | .023 | 4.03 | 1.75 | .30 | .17 | 16.4 | 13 | 26 | .....do | N. O. Co. Vict. 12, exp. 8. |
| 50 | .136 | .032 | .240 | .018 | .022 | 7.75 | 1.77 | .24 | .13 | 12.5 | 12 | 10 | .....do | N. O. Co. Viv. Low. 3. |
| 51 | .146 | .038 | .315 | .028 | .023 | 5.28 | 2.17 | .26 | .12 | 11.8 | ..... | 32 | .....do | L. V. W. B., Oat. 7. |
| 52 | .142 | .038 | .274 | .021 | .032 | 6.80 | 1.93 | .27 | .14 | 13.1 | 13 | 19 | .....do | N. O. Co. Vict. 1 and 2. |
| 53 | .142 | .037 | .221 | .098 | .027 | 1.44 | 1.55 | .26 | .17 | 17.2 | 13 | 13 | .....do | N. O. Co. Vict. 20, exp. 7. |
| 54 | .144 | .030 | .259 | .038 | .018 | 3.82 | 1.81 | .21 | .12 | 10.8 | 12 | Trace. | .....do | N. O. Co. Eur. 12. |
| 55 | .143 | .032 | .246 | .028 | .024 | 5.20 | 1.72 | .23 | .13 | 12.7 | 12 | 3 | Thompson | N. O. Co. Viv., Low. 1. |
| 56 | .141 | .037 | .244 | .018 | .022 | 7.67 | 1.73 | .26 | .15 | 12.7 | 12 | 8 | .....do | N. O. Co. Viv., Low. 2. |
| 57 | .150 | .045 | .228 | .045 | .023 | 3.33 | 1.53 | .30 | .20 | 10.9 | 12 | 5 | Washington | N. O. Co. Viv., Low. 4. |
| 58 | .141 | .034 | .228 | .079 | .036 | 1.79 | 1.61 | .24 | .15 | 11.7 | 12 | 46 | .....do | N. O. Co. Flor. |
| 59 | .143 | .038 | .255 | .836 | .052 | .17 | 1.78 | .26 | .15 | 6.0 | ..... | 53 | .....do | Bryn Mawr. |
| 60 | .142 | .022 | .139 | .033 | .013 | 4.37 | .98 | .16 | .16 | 4.8 | ..... | Trace. | .....do | E. H., Redlands. |
| 61 | .156 | .033 | .289 | .019 | .019 | 8.16 | 1.85 | .21 | .12 | 11.8 | ..... | 58 | Thompson | L. V. W. B., Dix. |
| 62 | .153 | .044 | .250 | .071 | .023 | 2.16 | 1.63 | .29 | .18 | 17.4 | 13 | 1 | Washington | N. O. Co. Vict. 9. |
| 63 | .159 | .043 | .273 | .074 | .022 | 2.14 | 1.71 | .27 | .16 | 16.5 | 13 | 15 | .....do | N. O. Co. Vict. 20, exp. 2. |
| 64 | .156 | .034 | .243 | .029 | .018 | 5.35 | 1.55 | .22 | .14 | 15.3 | 12 | Trace. | .....do | N. O. Co. Eur. 9. |
| 65 | .154 | .034 | .260 | .047 | .029 | 3.30 | 1.69 | .22 | .13 | 10.5 | 12 | 50 | .....do | N. O. Co. Spring 2. |
| 66 | .152 | .027 | .201 | .018 | .026 | 8.37 | 1.32 | .18 | .14 | 12.1 | ..... | 56 | .....do | Vivi. |
| 67 | .159 | .036 | .278 | .147 | .031 | 1.08 | 1.75 | .22 | .13 | 16.6 | ..... | 67 | .....do | Colton Ave. Sm. |
| 68 | .169 | .041 | .291 | .053 | .026 | 3.20 | 1.73 | .24 | .14 | 11.5 | ..... | 44 | .....do | L. V. W. B., Oat. 4. |
| 69 | .162 | .028 | .193 | .023 | .021 | 6.94 | 1.19 | .17 | .14 | 9.7 | ..... | 42 | .....do | Viv. |
| 70 | .165 | .043 | .265 | .562 | .047 | .29 | 1.60 | .26 | .16 | 8.7 | ..... | 95 | .....do | Bryn Mawr. |
| 71 | .163 | .047 | .309 | .045 | .027 | 3.62 | 1.89 | .29 | .15 | 12.4 | ..... | 59 | .....do | Bryn Mawr. |
| 72 | .162 | .046 | .220 | .133 | .022 | 1.23 | 1.36 | .28 | .21 | 13.5 | ..... | 8 | .....do | Redlands. |
| 73 | .177 | .055 | .248 | .132 | .013 | 1.35 | 1.39 | .31 | .22 | 15.4 | 13 | 1 | .....do | N. O. Co. Vict. 15. |
| 74 | .174 | .045 | .230 | .148 | .027 | 1.17 | 1.32 | .26 | .19 | 17.2 | 13 | 67 | .....do | N. O. Co. Vict. 20, exp. 1. |
| 75 | .171 | .042 | .260 | .104 | .023 | 1.64 | 1.52 | .24 | .16 | 16.6 | 13 | 12 | .....do | N. O. Co. Vict. 20, exp. 4. |
| 76 | .178 | .047 | .245 | .842 | .052 | .22 | 1.38 | .27 | .19 | 8.9 | ..... | 12 | .....do | Redlands. |
| 77 | .186 | .048 | .270 | .031 | .013 | 6.03 | 1.45 | .26 | .18 | 12.9 | ..... | 96 | .....do | Palm Ave., Redl. |
| 78 | .188 | .037 | .195 | .003 | .014 | 56.50 | 1.04 | .20 | .19 | ..... | ..... | 99 | .....do | Bloomington. |
| 79 | .182 | .040 | .350 | .020 | .025 | 8.95 | 1.93 | .22 | .11 | 14.1 | ..... | 64 | Valencia | L. V. W. B., Dix. |
| 80 | .184 | .032 | .250 | .026 | .022 | 6.46 | 1.35 | .17 | .13 | 9.3 | 12 | 40 | Washington | N. O. Co. Spring 1. |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 81 | .190 | .044 | .268 | .027 | .032 | 7.15 | 1.40 | .23 | .16 | 15.4 | 13 | Trace. | Thompson..... | N. O. Co., Vict. 14. |
| 82 | .198 | .040 | .282 | .028 | .029 | 6.30 | 1.41 | .20 | .14 | 15.0 | 13 | 1 | .....do..... | N. O. Co., Vict. 16. |
| 83 | .195 | .056 | .368 | .030 | .022 | 6.55 | 1.88 | .30 | .15 | 10.6 | 12 | 32 | .....do..... | N. O. Co., Viv. Up. 2. |
| 84 | .194 | .038 | .260 | .031 | .028 | 6.45 | 1.34 | .19 | .15 | 13.2 | 12 | 13 | Washington ... | N. O. Co. Viv. Up. 6. |
| 85 | .198 | .033 | .223 | .034 | .028 | 5.83 | 1.02 | .16 | .15 | 12.7 | 12 | 14 | .....do..... | N. O. Co., Viv. Low. 8. |
| 86 | .209 | .031 | .223 | .012 | .023 | 17.80 | 1.11 | .15 | .14 | 8.4 | ..... | 28 | Grapefruit..... | L. V. W. B., Dix. |
| 87 | .207 | .047 | .273 | .033 | .013 | 6.26 | 1.32 | .23 | .17 | 11.9 | ..... | 94 | Navel..... | Citrus Ave. Redl'ds. |
| 88 | .212 | .050 | .274 | .024 | .016 | 8.80 | 1.29 | .23 | .18 | 12.7 | ..... | 36 | .....do..... | Citrus Ave. Redl'ds. |
| 89 | .215 | .030 | .177 | .019 | .018 | 11.20 | .82 | .14 | .17 | ..... | ..... | 2 | .....do..... | Bloomington. |
| 90 | .211 | .033 | .226 | .009 | .019 | 23.00 | 1.07 | .16 | .15 | 12.0 | 12 | Trace. | Washington.... | N. O. Co., Eur. 6. |
| 90a | .218 | .038 | .288 | .018 | .023 | 11.84 | 1.32 | .18 | .13 | 13.0 | ..... | 65 | .....do..... | L. V. W. B., Dix. |
| 91 | .220 | .039 | .253 | .031 | .030 | 7.30 | 1.16 | .18 | .16 | 11.7 | 12 | 32 | Grapefruit..... | N. O. Co., Viv. Up. 3. |
| 92 | .210 | .030 | .266 | .019 | .028 | 10.95 | 1.26 | .14 | .11 | 11.5 | 12 | 17 | Washington.... | N. O. Co., Viv. Up. 5. |
| 93 | .221 | .050 | .299 | .041 | .023 | 5.18 | 1.35 | .22 | .17 | 13.8 | ..... | 4 | Navel..... | Citrus Ave. Redl'ds. |
| 94 | .233 | .038 | .222 | .010 | .018 | 23.00 | .95 | .16 | .17 | 9.1 | ..... | 3 | Washington.... | Rod., Highland. |
| 95 | .239 | .050 | .358 | .795 | .055 | .30 | 1.49 | .21 | .14 | 10.6 | ..... | Trace. | Navel..... | Mull., Bryn Mawr. |
| 96 | .236 | .039 | .331 | .021 | .025 | 11.25 | 1.40 | .17 | .12 | 10.3 | ..... | 9 | Washington.... | L. V. W. B., Oat. 6. |
| 97 | .232 | .030 | .278 | .018 | .032 | 12.65 | 1.18 | .13 | .11 | 11.3 | 12 | 14 | Thompson..... | N. O. Co., Viv. Up. 4. |
| 98 | .245 | .044 | .284 | .024 | .021 | 10.10 | 1.16 | .18 | .15 | 11.8 | ..... | 46 | Washington.... | Thomp., Highland. |
| 99 | .241 | .037 | .200 | .014 | .012 | 17.00 | .83 | .15 | .18 | 8.4 | ..... | None. | .....do..... | Lin., Highland. |
| 100 | .244 | .024 | .134 | .017 | .017 | 14.60 | .55 | .10 | .18 | 4.7 | ..... | 89 | Navel..... | Men. |
| 101 | .271 | .035 | .266 | .338 | .032 | .81 | .98 | .13 | .13 | 7.1 | ..... | 61 | Washington.... | A. E. F., Highland. |
| 102 | .279 | .028 | .240 | .021 | .018 | 13.40 | .86 | .10 | .12 | 6.3 | ..... | 64 | Navel..... | Azusa. |
| 103 | .282 | .027 | .199 | .073 | .019 | 3.89 | .71 | .10 | .14 | 8.9 | ..... | 5 | ....do..... | G. C. L., Highland. |
| 104 | .287 | .034 | .277 | .011 | .028 | 26.40 | .97 | .12 | .12 | 13.4 | 12 | Trace. | Thompson..... | N. O. Co., Eureka 5. |
| 105 | .302 | .041 | .265 | .021 | .013 | 14.40 | .88 | .13 | .15 | 10.0 | ..... | 39 | Washington.... | L. V. W. B., Oat 1. |
| 106 | .313 | .031 | .207 | .074 | .023 | 4.20 | .66 | .10 | .15 | 8.1 | ..... | 33 | Valencia..... | Fr., Sun. Mt. |
| 107 | .333 | .049 | .408 | .105 | .028 | 3.17 | 1.22 | .15 | .12 | 9.2 | ..... | 90 | Navel..... | Azusa. |
| 108 | .323 | .042 | .312 | .011 | .018 | 29.80 | .97 | .13 | .13 | 7.9 | ..... | 12 | Washington.... | L. V. W. B., Oat. 3. |
| 109 | .342 | .036 | .286 | .008 | .015 | 45.10 | .84 | .10 | .13 | 7.7 | ..... | 12 | Grapefruit..... | L. V. W. B., Sun. Mt. |
| 110 | .346 | .027 | .273 | .034 | .016 | 10.10 | .79 | .08 | .10 | 9.7 | ..... | 5 | Navel..... | Phy., Highland. |
| 111 | .342 | .038 | .310 | .194 | .017 | 1.76 | .88 | .11 | .12 | 10.7 | ..... | 53 | Washington.... | R. S. T., Highland. |
| 112 | .353 | .040 | .281 | .035 | .013 | 10.10 | .80 | .11 | .14 | 9.1 | ..... | 76 | Navel..... | Fl., Highland. |
| 113 | .373 | .040 | .288 | .164 | .023 | 2.28 | .77 | .11 | .14 | 9.8 | ..... | 71 | .....do..... | A. E. F., Highland. |
| 114 | .378 | .033 | .282 | .009 | .014 | 42.00 | .75 | .09 | .12 | 8.9 | ..... | 70 | .....do..... | Azusa. |
| 115 | .372 | .026 | .300 | .012 | .015 | 32.00 | .80 | .07 | .09 | 7.5 | ..... | 10 | Valencia..... | L. V. W. B., Sun. Mt. |
| 116 | .425 | .043 | .347 | .016 | .010 | 27.00 | .82 | .10 | .13 | 8.7 | ..... | None. | Nursery..... | R. S. T., Highland. |
| 117 | .431 | .045 | .499 | .012 | .022 | 37.00 | 1.15 | .10 | .09 | 10.9 | ..... | 23 | Navel..... | Pomona. |
| 118 | .474 | .041 | .462 | .026 | .012 | 18.30 | .98 | .09 | .09 | 8.1 | ..... | None. | .....do..... | Lock., Highland. |
| 119 | .503 | .035 | .427 | .025 | .018 | 20.00 | .85 | .07 | .08 | 10.8 | ..... | 8 | .....do..... | Rod., Highland. |
| 120 | .506 | .042 | .332 | .011 | .015 | 46.50 | .66 | .08 | .13 | 8.7 | ..... | 12 | Washington.... | L. V. W.. B., Oat. 2. |
| 121 | .534 | .052 | .522 | .033 | .017 | 16.00 | .98 | .10 | .10 | 13.0 | ..... | None. | Navel..... | Ontario. |
| 122 | .557 | .052 | .589 | .018 | .013 | 30.60 | 1.05 | .09 | .09 | 7.9 | ..... | None. | .....do..... | Upland. |
| 123 | .584 | .038 | .287 | .115 | .018 | 5.10 | .49 | .07 | .13 | 10.8 | ..... | 9 | Washihgton.... | R. S. T., Highland. |
| 124 | .639 | .040 | .331 | .057 | .025 | 11.20 | .52 | .06 | .12 | 7.1 | ..... | 19 | Navel..... | Azusa. |
| 125 | .670 | .060 | .516 | .107 | .021 | 6.30 | .77 | .09 | .12 | 15.0 | ..... | None. | Valencia..... | Do. |
| 126 | .695 | .067 | .628 | .015 | .022 | 46.20 | .91 | .10 | .11 | 21.2 | ..... | Trace. | Navel..... | Do. |
| 127 | .769 | .037 | .298 | .036 | .012 | 22.40 | 3.87 | .05 | .12 | 9.7 | ..... | 90 | .....do..... | Pomona. |

The results of the analyses of orange soils, together with the percentage of mottled leaves in each grove, are presented in Table I. In all cases, unless otherwise stated, the soil data given represent a sample 3 feet in depth. Each foot section to a depth of 3 feet was analyzed separately, but the results of the determinations on the individual foot sections disclose no relationships that are not equally well represented by the mean value. The analytical data from the orange groves were first considered in relation to soil type. While the soils around Redlands, Highlands, and Riverside differ to some extent in their physical characteristics, no correlation between mottle-leaf and soil type was in evidence. Furthermore, the fact that mottling seems to be about equally advanced on all the soil types of this area, other conditions, as age of grove, general treatment, etc., being the same, would indicate prima facie that the soil type is by no means a controlling factor. The results obtained from all the orange groves studied in the districts around Riverside, Redlands, Highland, and Rialto are therefore presented collectively. A few groves studied around Pomona, Ontario, and Azusa are not included in this grouping, since the soil conditions of these districts are quite different, in so far at least as the organic content is concerned.

To facilitate further comparison, the orange-grove data are grouped in Table II on the basis of the percentage of mottling. Each group represents the average of about 20 groves, so that each point on the accompanying graphs represents an average of about 60 separate determinations of a given factor, and approximately 200 mottling determinations.[1] The fact that mottling is not dependent upon the texture of the soil is again emphasized in this table, which shows that the moisture retentiveness of the several groups as measured by the moisture equivalent[2] is very nearly the same.

TABLE II.—*Analysis of orange-grove soils near Riverside, Redlands, Highland, and Rialto, Cal., grouped according to percentage of mottled leaves, each group containing approximately 20 groves*

| Group. | Percentage of— | | | | | Ratio of— | | | | Moisture equivalent. | Mottled leaves. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Humus. | Total nitrogen. | Organic carbon. | Mineral carbonates. | Mineral bicarbonates. | Humus to lime. | Carbon to humus. | Nitrogen to humus. | Nitrogen to carbon. | | |
| | | | | | | | | | | *Per cent.* | *Per cent.* |
| 1..... | 0.119 | 0.036 | 0.237 | 0.069 | 0.023 | 1.72 | 2.50 | 0.303 | 0.152 | 11.3 | 88 |
| 2..... | .142 | .036 | .256 | .066 | .024 | 2.15 | 1.54 | .254 | .141 | 12.4 | 64 |
| 3..... | .170 | .039 | .254 | .093 | .026 | 1.83 | 1.67 | .229 | .154 | 11.6 | 43 |
| 4..... | .165 | .039 | .255 | .080 | .027 | 2.06 | 1.65 | .237 | .153 | 12.6 | 19 |
| 5..... | .244 | .039 | .261 | .068 | .020 | 3.59 | 1.93 | .159 | .149 | 11.9 | 8 |
| 6..... | .204 | .038 | .263 | .079 | .028 | 2.58 | 1.78 | .186 | .144 | 12.8 | 1 |

[1] The ratios in Tables II and IV are calculated from the mean values of the measured factors.

[2] The moisture equivalent is a measure of the moisture retentiveness of a soil, and is numerically equal to the percentage of moisture which a given soil is able to retain in opposition to a centrifugal force 1,000 times that of gravity. The finer the soil particles the greater is the moisture equivalent.

RELATION OF "HUMUS" IN SOIL TO LEAF MOTTLING OF ORANGES

The relation of the percentage of leaf mottling to the percentage of humus in the soil is shown in figure 1, the humus being plotted as the abscissas and the mottling as ordinates. While the points by no means form a smooth curve, there is a very evident inverse relation, showing that a high humus content is correlated with a low percentage of mottling.

As already mentioned, some time is required for an orange or lemon tree to respond to an application of manure. Consequently, in cases where manure has been recently added to a grove a measurable increase in humus may result without sufficient time having elapsed for a leaf response. Furthermore, when a leaf is well advanced in mottling, it does not recuperate (except by special leaf treatment), but remains mottled until it drops. Hence, a new set of leaves must be grown before the mottling will disappear from the tree, although the first stage of mottling, especially in a young leaf, may disappear as the leaf grows, if conditions become favorable. With these facts in mind, one would expect that the humus graph presented in figure 1 would show some inconsistencies, especially since other soil factors besides humus undoubtedly influence the nutrition of the tree.

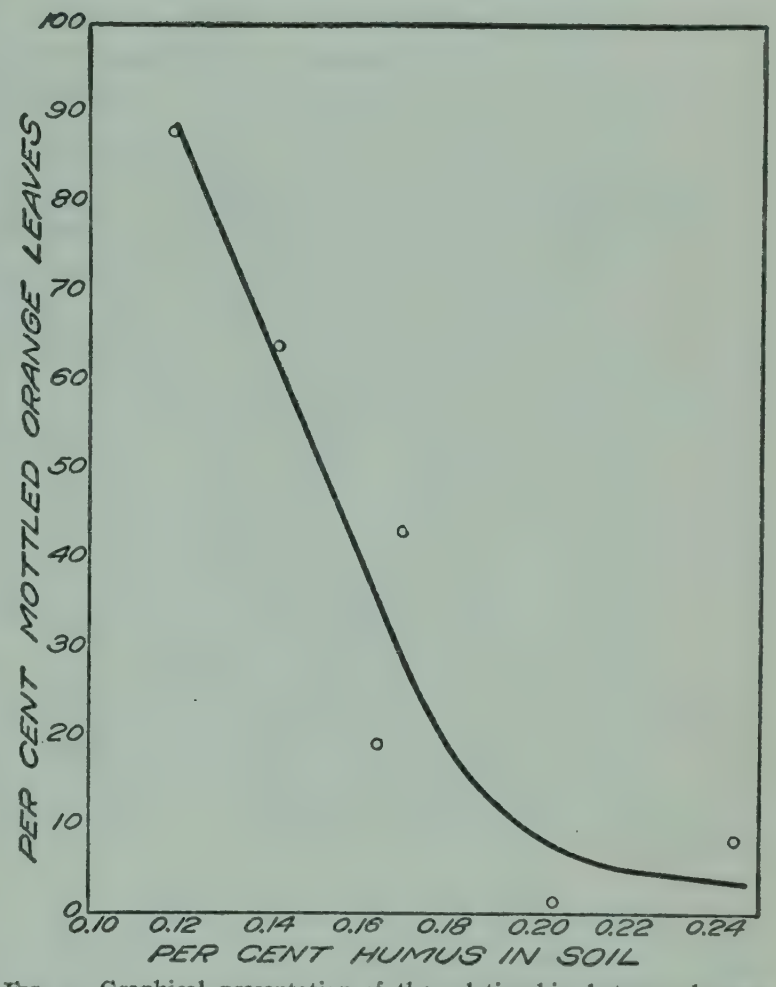



FIG. 1.—Graphical presentation of the relationship between humus content of soil and percentage of mottled orange leaves (from data in Table II).

The relationship between humus and mottling has been examined in more detail by the use of statistical methods. The form of the graph in figure 1 suggests an approximate hyperbolic relationship between humus content and mottling. In order to reduce the data to a suitable linear form for calculating the coefficient of correlation, the reciprocal of the humus content of each soil was calculated—that is, the number of grams

of soil required to include 1 gm. of humus. The coefficient of correlation between this quantity and the percentage of mottled leaves for all the orange groves included in the main group was found to be $0.67 \pm 0.03$. The association would be represented by the square of this quantity, or 0.45. In other words, approximately one-half of the mottling can be accounted for by the low humus content of the soil. This conclusion is reached from a consideration of the data by impartial statistical methods and is free from personal bias.

The failure of the trees in some cases to respond to manure appears to be due to the methods of cultivation and irrigation employed. It has been the general practice in California orange culture to maintain a deep dust mulch in the groves by cultivating frequently during the summer months. In fact, the cultivation which is carried on between irrigation periods, combined with the opening and closing of irrigation furrows, results in working the surface soil on an average of nearly once a week during the summer months. It is quite impossible for any effective root development to take place in this surface layer under such conditions. The roots are destroyed by the constant cultivation; and owing to the frequent stirring, the soil during the greater part of the time is entirely too dry for root development. Yet this is the part of the soil to which manure is applied, the usual practice being to disk the manure into the mulch. Even when the manure or a cover crop is plowed under, the plowing is often so shallow that the material turned under is within reach of the teeth of the cultivator. The result is, therefore, that the organic matter is partly disintegrated and lost without ever coming in contact with the feeding roots of the tree. Under such conditions it is not surprising that little benefit has resulted from the use of manure. It would be difficult to conceive a more effective method for the rapid destruction of the organic matter than the repeated stirring, moistening, and drying to which it is subjected in this deep surface mulch.

The difference in humus content between the soils of the badly mottled groves and those relatively free from mottling is about 0.1 per cent. This difference may at first sight appear small; but when expressed in terms of the weight of the soil, its magnitude becomes apparent. An acre of soil 3 feet in depth weighs approximately 10,000,000 pounds, so that a humus content of one-tenth of 1 per cent is equivalent to 10,000 pounds of humus. Data regarding the amount of humus formed from a ton of organic matter are not at present available, but manure would probably not often yield more than 10 per cent, or 200 pounds of "humus", or "*matière noire*", per ton. On the basis of this assumption it would require an application of at least 50 tons of manure per acre to bring the humus content of the badly mottled groves up to that of the groves relatively free from mottling.

Mention has already been made of the fact that the appearance of the mottled leaves indicates that the mottled Citrus tree is failing to secure something essential in the formation of chlorophyll. The association between mottling and low humus suggests that the missing substance may be some organic compound normally formed during the decomposition of organic matter in the soil or associated with the formation of humus, in which event the "humus" content would be indicative to some extent of the amount of this substance formed. Until further information is available in this connection, practical considerations point to the immediate enrichment of the humus content of the soil as the most promising specific for mottle-leaf.

### RELATION OF MINERAL CARBONATES TO MOTTLING OF ORANGE TREES

The mineral carbonates in the soils of the area studied consist for the most part of calcium carbonate (limestone). The percentage is usually low (see Table I), although large deposits of limestone are found in some of the hills rising from the floor of the valley. No significant correlation was found to exist between the percentage of mineral carbonates and the percentage of mottled leaves (correlation coefficient $= 0.07 \pm 0.06$). In other words, there is no evidence that the amount of mineral carbonates within the limits found in these soils bears any relation to mottling. Most of the groves in the areas studied have not been limed, and where lime has been used, the amount applied has with few exceptions been so small as to be negligible in the determinations. For example, an application of a ton of limestone per acre would mean an increase of only two one-hundredths per cent when calculated on the weight of the soil to a depth of 3 feet. The effect of heavy applications of lime on mottling has not yet been definitely settled by properly controlled field experiments. This matter should furthermore not be confused with the evident beneficial effect of lime in improving the physical condition of some of the soils in the area studied.

### RATIO OF HUMUS TO MINERAL CARBONATES AS AFFECTING MOTTLING OF ORANGE TREES

The ratio of humus to mineral carbonates in orange groves is plotted in figure 2 against the percentage of mottling. While the relationship is not marked, the mottling tends to diminish as the humus-lime ratio increases. The correlation between the reciprocal of this ratio and the mottling was computed and found to be $0.17 \pm 0.06$. Since no relationship was observed between the lime content of the soil and the percentage of mottling, it seems probable that the correlation observed in the case of the humus-lime ratio is dependent wholly on the humus correlation. The result indicates that the humus content of the soil should be taken

into consideration in applying lime and that such treatment would be more likely to be beneficial in the case of soils with a high humus content.

### RELATION OF ORGANIC CARBON TO MOTTLING OF ORANGE LEAVES

The correlation between the total organic carbon in the soil and the leaf mottling is very low ($-0.10 \pm 0.06$). Organic matter is not effective in nutrition until decomposition has set in, and the results indicate that the amount of those decomposition products effective in the control of mottle-leaf and available in the soil at a given time is not necessarily proportional to the total organic carbon present. The negative sign of the correlation coefficient shows that the mottling tends to decrease as the organic matter increases.

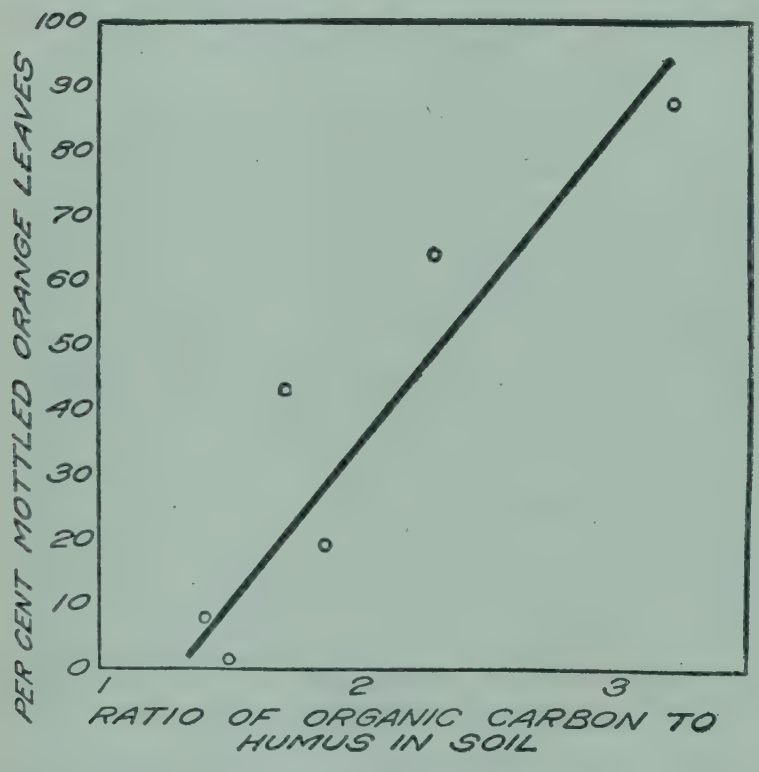



FIG. 2.—Graphical presentation of the relationship between the ratio of organic carbon to humus in the soil and the percentage of mottled orange leaves (from data in Table II).

### RATIO OF ORGANIC CARBON TO HUMUS IN RELATION TO MOTTLING OF ORANGE LEAVES

The data presented in Table II for the six groups of orange groves show that an increase in the ratio of organic carbon to humus is accompanied by an increase in mottling (fig. 3). This relationship may be partly due to the fact that the mean organic content of the soils of the several groups is nearly the same throughout, although the reduction in mottling is accompanied by a slight increase in organic carbon.

A correlation of $0.43 \pm 0.06$ was found between the organic carbon-humus ratio and the percentage of mottling. While this correlation may be partly associative, as in the case of the lime-humus ratio, the results indicate that it is important in the nutrition of the orange tree that the organic matter be decomposed, so far as possible, into humus, since the greater the proportion of humified organic matter, the smaller the percentage of mottling. This, of course, does not necessarily indicate that what we term "humus" is the most effective form of organic matter for promoting a healthy growth of orange leaves; but if a

soil can properly humify organic matter, the latter will apparently go through the decomposition stages most beneficial to the growth of the tree.

### NITROGEN CONTENT AND MOTTLING

The total nitrogen content in the soil was surprisingly uniform regardless of grove conditions and soil types. The variation in total nitrogen within the limits found in the soils of the groves examined appears to bear no relation to the percentage of mottling (correlation coefficient $= -0.02 \pm 0.06$). A part of the nitrogen is undoubtedly held in a form not immediately available to the tree and in this respect is somewhat analogous to the total organic carbon in the soil. When the orange soils are grouped on the basis of mottling, as in Table II, the two most badly mottled groups show the lowest average nitrogen content, but the differences are so small as to have little significance. The other groups will be seen from Table II to have practically the same average nitrogen content. Such relationship as exists may be due to the fact that part of the total nitrogen is combined as "humus," so that the low humus soils would be lower in nitrogen.[1]

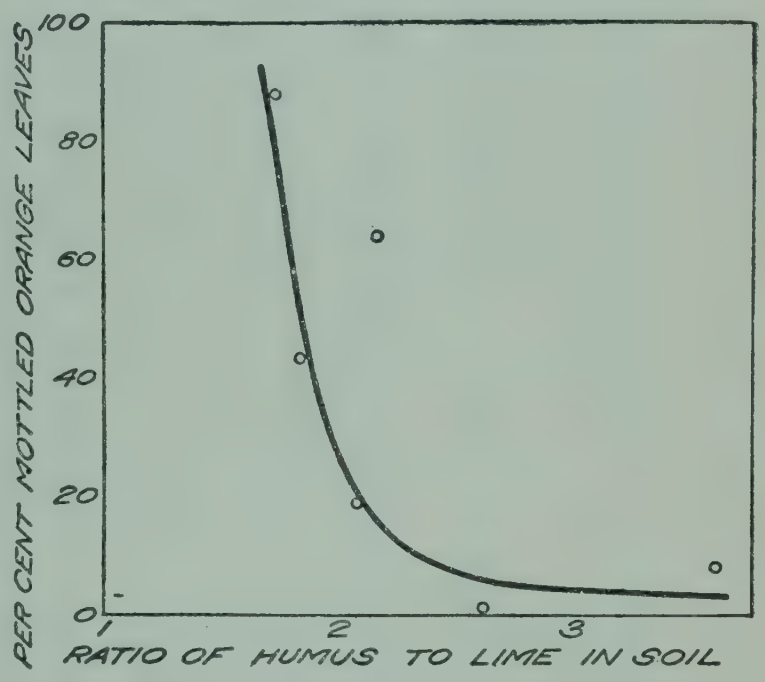



FIG. 3.—Graphical presentation of the relationship between the ratio of humus to lime in the soil and the percentage of mottled leaves (from data in Table II).

### LEMON SOILS

The field studies of the lemon groves were carried on in a manner similar to that of the orange groves. The lemon groves selected all belong to the same company and constitute the principal lemon groves in the Riverside district. The data for the individual groves are presented in Table III and are grouped on the basis of mottling in Table IV.

[1] For data regarding the nitrate content of the soils of the Riverside area, see Kellerman, K. F., and Wright, R. C. Relation of bacterial transformations of soil nitrogen to nutrition of citrous plants. *In* Jour. Agr. Research, v. 2, no. 2, p. 101-113, 7 fig. 1914.

TABLE III.—*Analyses of lemon-grove soils in California to a depth of 3 feet*

| No. | Percentage of— | | | | | Ratio of— | | | | Moisture equivalent. | Trees. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Humus. | Total nitrogen. | Organic carbon. | Carbonates. | Bicarbonates. | Humus to lime. | Carbon to humus. | Nitrogen to humus. | Nitrogen to carbon. | | Age. | Percentage of mottled leaves. | Location. |
| 1.. | 0.044 | 0.031 | 0.265 | 0.041 | 0.028 | 1.08 | 6.00 | 0.70 | 0.12 | 11.5 | 20 | 88 | A. H. 32 –36 |
| 2.. | .045 | .035 | .275 | .058 | .025 | .77 | 6.14 | .78 | .13 | 10.5 | 20 | 93 | A. H. 32 –46 |
| 3.. | .047 | .031 | .260 | .069 | .026 | .68 | 5.48 | .68 | .12 | 12.6 | 21 | 44 | A. H. 80 – 6 |
| 4.. | .058 | .030 | .215 | .032 | .019 | 1.81 | 3.72 | .53 | .14 | 6.8 | 20 | 68 | A. H. 32 –49 |
| 5.. | .059 | .028 | .214 | .090 | .023 | .65 | 3.62 | .48 | .13 | 9.5 | 20 | 79 | A. H. 25½– 8 |
| 6.. | .057 | .033 | .263 | .197 | .033 | .29 | 4.65 | .58 | .13 | 12.0 | 21 | 76 | A. H. 56 – 6 |
| 7.. | .058 | .034 | .275 | .058 | .026 | 1.01 | 4.80 | .59 | .13 | 12.8 | 23 | 85 | A. H. 46 – 6 |
| 8.. | .069 | .031 | .212 | .075 | .025 | .92 | 3.10 | .46 | .15 | 11.0 | 9 | 59 | A. H. 32 –28 |
| 9.. | .066 | .032 | .234 | .063 | .026 | 1.04 | 3.58 | .49 | .14 | 10.2 | 21 | 89 | A. H. 32 –43 |
| 10.. | .068 | .028 | .244 | .054 | .025 | 1.25 | 3.60 | .42 | .12 | 11.4 | 21 | 89 | A. H. 32 –54 |
| 11.. | .066 | .039 | .249 | .077 | .029 | .66 | 3.80 | .59 | .16 | 13.2 | 21 | 59 | A. H. 80 – 4 |
| 12.. | .069 | .045 | .295 | .045 | .024 | 1.54 | 4.26 | .65 | .15 | 15.1 | 23 | 81 | A. H. 45 – 8 |
| 13.. | .070 | .032 | .244 | .077 | .024 | .91 | 3.52 | .47 | .13 | 9.8 | 23 | 93 | A. H. 32 – 7 |
| 14.. | .066 | .032 | .236 | .027 | .021 | 2.40 | 3.55 | .49 | .18 | 11.0 | 23 | 91 | A. H. 32 –14 |
| 15.. | .064 | .029 | .226 | .075 | .023 | .86 | 3.54 | .45 | .13 | 8.3 | 21 | 90 | A. H. 17 – 1 |
| 16.. | .067 | .029 | .258 | .053 | .026 | 1.26 | 3.85 | .43 | .11 | 12.0 | 21 | 86 | A. H. 56 – 4 |
| 17.. | .069 | .023 | .280 | .166 | .041 | .41 | 4.10 | .34 | .08 | 13.7 | 21 | 68 | A. H. 56 – 1 |
| 18.. | .062 | .031 | .278 | .075 | .030 | .81 | 4.47 | .50 | .11 | 11.8 | 21 | 76 | A. H. 46 – 5 |
| 19.. | .068 | .036 | .265 | .086 | .025 | .81 | 3.90 | .53 | .14 | 11.3 | 21 | 76 | A. H. 18 – 4 |
| 20.. | .070 | .026 | .316 | .089 | .031 | .67 | 4.55 | .37 | .08 | 12.0 | 21 | 83 | A. H. 54 – 1 |
| 21.. | .072 | .029 | .243 | .086 | .024 | .84 | 3.36 | .38 | .12 | 9.6 | 21 | 94 | A. H. 32 –44 |
| 22.. | .074 | .031 | .242 | .066 | .022 | 1.11 | 3.28 | .43 | .13 | 13.6 | 21 | 56 | A. H. 80 – 3 |
| 23.. | .079 | .030 | .228 | .032 | .019 | 2.48 | 2.88 | .38 | .13 | 8.2 | 21 | 89 | A. H. 25½– 6 |
| 24.. | .076 | .029 | .221 | .041 | .014 | 1.85 | 2.91 | .38 | .13 | 9.7 | 21 | 94 | A. H. 17 – 3 |
| 25.. | .071 | .043 | .286 | .244 | .038 | .29 | 4.04 | .60 | .15 | 14.9 | 21 | 70 | [a]A. H. 56 – 7 |
| 26.. | .071 | .037 | .282 | .122 | .034 | .58 | 3.96 | .52 | .13 | 13.8 | 21 | 56 | A. H. 56 – 2 |
| 27.. | .071 | .033 | .278 | .097 | .038 | .71 | 3.82 | .47 | .12 | 13.8 | 23 | 67 | A. H. 46 – 4 |
| 28.. | .078 | .033 | .282 | .138 | .033 | .57 | 3.63 | .42 | .12 | 11.5 | 19 | 80 | A. H. A3 – 3 |
| 29.. | .083 | .039 | .202 | .029 | .032 | .84 | 2.44 | .47 | .19 | 13.3 | 9 | 72 | A. H. 32 –21 |
| 30.. | .081 | .028 | .197 | .028 | .030 | 2.92 | 2.44 | .35 | .14 | 7.0 | 21 | 77 | A. H. 32 –51 |
| 31.. | .082 | .043 | .247 | .098 | .033 | .82 | 3.00 | .53 | .18 | ..... | 21 | 85 | A. H. 74 – 3 |
| 32.. | .089 | .028 | .190 | .028 | .021 | 3.24 | 2.12 | .32 | .15 | 6.9 | 21 | 82 | A. H. 32 –50 |
| 33.. | .082 | .043 | .240 | .098 | .033 | .82 | 2.92 | .53 | .18 | ..... | 21 | 46 | A. H. 74 – 2 |
| 34.. | .089 | .027 | .274 | .033 | .024 | 2.72 | 3 04 | .30 | .10 | 10.8 | 21 | 86 | A. H. 80 – 1 |
| 35.. | .089 | .036 | .272 | .083 | .024 | 1.08 | 3.06 | .40 | .13 | 7.4 | 21 | 59 | [b]A. H. 56 – 7 |
| 36.. | .081 | .041 | .265 | .084 | .028 | .97 | 3.26 | .50 | .15 | 12.9 | 21 | 85 | A. H. 53 – 2 |
| 37.. | .092 | .028 | .239 | .018 | .021 | 5.00 | 2.60 | .30 | .12 | 9.8 | 21 | 82 | A. H. 25½– 4 |
| 38.. | .094 | .028 | .190 | .033 | .019 | 2.80 | 2.03 | .30 | .15 | 8.4 | 21 | 79 | A. H. 25½– 2 |
| 39.. | .097 | .043 | .315 | .074 | .038 | 1.31 | 3.25 | .45 | .14 | 14.2 | 21 | 82 | A. H. 45 –10 |
| 40.. | .103 | .033 | .238 | .049 | .023 | 2.10 | 2.30 | .32 | .14 | 8.6 | 21 | 87 | A. H. 18 – 3 |
| 41.. | .117 | .046 | .230 | .087 | .030 | 1.16 | 1.97 | .40 | .20 | 13.0 | 23 | 84 | A. H. 45 – 9 |
| 42.. | .127 | .036 | .258 | .048 | .019 | 2.78 | 2.04 | .28 | .14 | 7.8 | 20 | 88 | A. H. 32 –52 |

[a] Upper. [b] Lower.

TABLE IV.—*Analyses of California lemon-grove soils, grouped according to percentage of mottled leaves, each group including eight groves*

| Group. | Percentage of— | | | | | Ratio of— | | | | Moisture equivalent. | Mottled leaves. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Humus. | Total nitrogen. | Organic carbon. | Mineral carbonates. | Mineral bicarbonates. | Humus to lime. | Carbon to humus. | Nitrogen to humus. | Nitrogen to carbon. | | |
| | | | | | | | | | | *Per cent.* | *Per cent.* |
| 1..... | 0.066 | 0.036 | 0.241 | 0.062 | 0.023 | 1.06 | 2.74 | 0.545 | 0.149 | 10.1 | 92 |
| 2..... | .081 | .033 | .258 | .050 | .024 | 1.64 | 3.18 | .407 | .128 | 10.6 | 87 |
| 3..... | .087 | .037 | .265 | .073 | .029 | 1.19 | 3.05 | .425 | .139 | 11.8 | 82 |
| 4..... | .072 | .033 | .237 | .097 | .029 | .74 | 3.29 | .458 | .139 | 11.0 | 76 |
| 5..... | .070 | .033 | .253 | .089 | .029 | .79 | 3.61 | .471 | .126 | 11.8 | 58 |

RELATION OF HUMUS IN THE SOIL TO MOTTLING OF THE LEMON LEAVES

It was found that the humus content of the soil in the orange groves varied inversely with the leaf mottling. In the case of the lemon groves no definite relation appears at first sight to exist between these two factors. However, a comparison of Table II with Table IV shows that the humus content of most of the orange groves was much higher than that of the lemon groves. It will be noted from Table IV that the humus content of the lemon-grove groups was in every case less than one-tenth of 1 per cent, an extremely low value. Orange groves in which the humus content approximates 0.1 per cent (Table II) show as high a percentage of mottling as the lemon groves. It would therefore appear that the humus content in the lemon groves is less than is necessary for the growth of a leaf comparatively free from mottling, assuming that lemon leaves would mottle to the same extent as orange leaves under the same conditions.

RELATION OF MINERAL CARBONATES IN THE SOIL TO LEAF MOTTLING

An indication of a slight relationship between the mineral carbonate content of the soil and the percentage of mottled leaves was obtained in the case of the lemon groves, as shown in figure 4. The correlation coefficient is $-0.31 \pm 0.09$, the minus sign indicating that the mottling decreases as the mineral carbonates (chiefly lime) increase. This correlation coefficient would indicate an association between mottling and lime content of about 10 per cent. The probable error is relatively so large that the result can be considered to be little more than indicative of the inverse character of the relationship. The percentage of mottling is very high even in the case of the highest lime content. The average amount of lime carbonate in the lemon-grove soils was about the same as in the orange-grove soils.

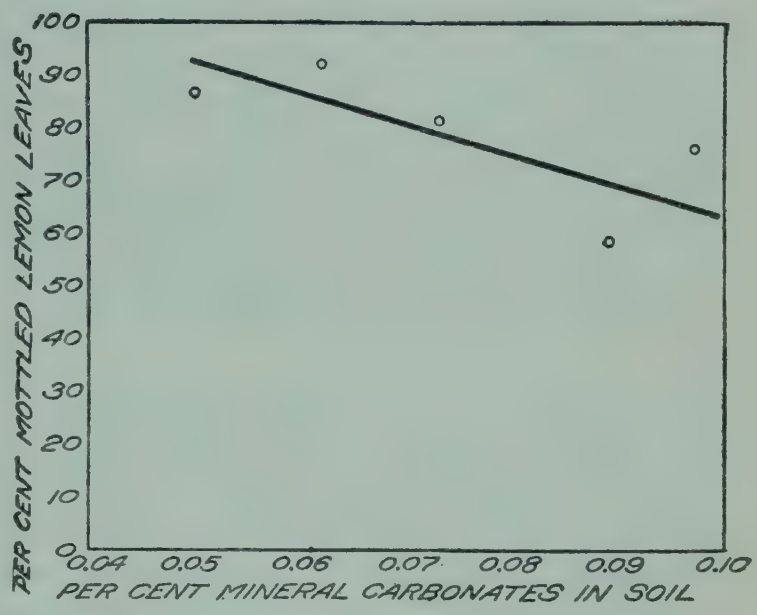



FIG. 4.—Graphical presentation of the relationship between the mineral carbonates in the soil and the percentage of mottled lemon leaves (from data in Table IV).

It is recognized that the lime-carbonate content is very low in all the soils represented in these areas and possibly a higher range of this constituent would bring out more definite results. The data presented are not sufficient to justify recommending the application of lime to lemon

groves which are low in this constituent, but lime experiments with the lemon would appear to be more promising than with the orange, other conditions being the same.

No correlation was found to exist between organic carbon or total nitrogen and mottling, the correlation coefficient in each case being no greater than the probable error.

## SUMMARY

Mottle-leaf of Citrus trees is characterized by the disappearance of chlorophyll from parts of the leaf, the portions farthest removed from the midrib and larger veins being first affected. As the disturbance progresses, the yellowish spots increase in size until the only remaining chlorophyll is confined to narrow areas along the midrib and the larger veins. The advanced stages are accompanied by a marked decrease in the size, quality, and yield of fruit. No organism has yet been proved to be causally associated with mottle-leaf, but the Citrus-root nematode has been found by Thomas to be widely distributed in mottled districts.

Mottle-leaf is found in most Citrus-fruit sections of California, but is more prevalent in some districts than in others. All the Citrus fruits grown in California are affected, including the Washington Navel, Thompson Improved Navel, and Valencia orange, grapefruit, tangerine, and lemon.

The conclusions of the present paper are based upon a field and laboratory study of 130 orange groves and 45 lemon groves, located mainly in Riverside and San Bernardino Counties, Cal. The percentage of mottled leaves was determined by examining 10 to 12 typical trees in each grove. A soil sample 3 feet in depth was taken near each tree, each foot sample being kept separately. These samples were analyzed for humus, organic carbon, mineral carbonates, bicarbonates, and total nitrogen.

During the earlier stages of mottling no serious reduction in yield was observed. The fruit yield was apparently not seriously reduced on either orange or lemon trees which had about 20 per cent of their leaves mottled. Sour-orange stock was found to induce more severe mottling in orange trees than sweet-orange stock, other conditions being the same. A mixed grove of Washington Navel and Valencia oranges showed no difference in the amount of mottling of these two varieties.

Badly mottled orange trees cut back and rebudded on the stumps produce badly mottled new top growth; and unless the soil treatment of such groves is changed, the mottling persists.

There was no noticeable difference in the amount of leaf mottling in groves on different soil types, other conditions being the same.

Orchards fertilized with organic substances, such as stable manure or cover crops plowed under, usually showed less mottling than groves supplied principally with commercial fertilizers. Groves which for some years had received only the "complete" fertilizers in general use in the

areas studied were badly mottled in all cases, so far as observed in these studies. This was also the case where sodium nitrate was used alone or as the principal fertilizer for some years.

The results of the soil analyses show in the case of oranges a marked inverse correlation between the humus content of the soil and the percentage of mottling, the latter tending to diminish as the humus content increases. An impartial statistical study of the data from the individual orange groves shows that approximately one-half the mottling can be accounted for by the low humus content of the soil.

The humus content of the lemon soils studied is much below that of most of the orange soils, averaging less than 0.1 per cent. This amount of humus is apparently too low to produce a normal foliage growth, all of the lemon groves being badly mottled.

No correlation was found between the mineral carbonates of the soil and the mottling of orange trees. In lemons the mottling decreased slightly as the mineral carbonates increased, but the correlation is low. The lime content of nearly all the Citrus soils studied is low, and the effect of heavy applications of lime can only be determined by suitably controlled field experiments. The present study indicates that the application of lime would be more likely to benefit lemon trees than orange trees.

The percentage of mottled leaves on orange trees is definitely correlated with the increase of the ratio of organic carbon to humus, indicating the importance of the organic matter in the soil being well decomposed.

No relation was found between the percentage of leaves mottled and the total nitrogen content in the soil in either the orange groves or the lemon groves studied.

The principal conclusion of this investigation is that the mottling of orange trees in the areas studied is definitely correlated with the low humus content of the soil, the mottling diminishing as the humus content increases. A study of the data by statistical methods shows that approximately one-half of the mottling can be accounted for on this basis. The incorporation of organic matter with the soil in such a manner as to be accessible to the roots during its decomposition is indicated as a promising treatment for mottle-leaf.

PLATE H

Various stages in mottle-leaf of the orange.

(740)

Shull

PLATE XCVI

Orange leaves showing mottle-leaf.

## PLATE XCVII

A more advanced stage of mottle-leaf of orange, showing the reduction in the size of the leaves.

# VEGETATIVE SUCCESSION UNDER IRRIGATION

By J. Francis Macbride [1]

*Agricultural Experiment Station of the University of Wyoming*

## INTRODUCTION

The data given in the following pages are compiled from observations made during the growing seasons of 1912, 1913, and 1914, on a ranch situated near Rock River, Albany County, Wyo., in the southeastern part of the State. The Union Pacific Railroad makes the altitude at the station 2,105 meters. As the part of the ranch with which we are concerned lies about 3 miles up Rock Creek, the altitude will approximate 2,134 meters, a figure that corresponds very well with the general elevation of the Laramie Plains (including the Laramie Basin), of which this is a section.

Several years ago the hay yield of the ranch, obtained entirely from the natural meadow lands, fell short of the consumption. Means were taken to increase the hay acreage by the transformation of bench land to meadow, a transformation accomplished simply by flooding during the successive growing seasons. This method has proved so successful that each succeeding year has seen greater and greater areas flooded until now large reservoirs (Pl. CI, fig. 2) are required to augment the water supply during the midsummer season.

Of course it takes several seasons to complete this change, and the following notes are the result of a study of the various phases of vegetation through which the bench land passes in this transition to meadow. At the time these observations were made (1913 and 1914) several adjoining tracts, each flooded for the first time in a different season, furnished an unusual opportunity for the comparison and study of their development from year to year and at the same time gave striking physical evidence of actual differences in the vegetation present. I refer to the varying tones of green which the different tracts assumed as the season advanced—a condition so marked that with a very little practice one could ride over the ranch and state authoritatively that this section has been under water for four years, this for two, and so on. Indeed, it was this beautiful blotching of the landscape with various shades of green and brown that led to the discovery of what was really happening.

[1] This work was done under the direction of Dr. Aven Nelson, of the Wyoming Experiment Station. If the conclusions, based on a study that was conceived in a spirit of helpfulness, prove of value, the credit can not be too largely assigned to the active interest and kindly encouragement of my adviser. The distressing difficulties under which the photographs were secured could not have been overcome except by Dr. Nelson's perseverance and constant help. I wish to thank Prof. A. S. Hitchcock, of the United States Department of Agriculture, for determining my material of the genus Agropyron; also Mr. V. H. Rowland, who furnished the determinations of the difficult genus Carex.

A collection of plants illustrating the salient features of the Rock Creek upland and meadow floras and substantiating the points brought out in this study is deposited in the Rocky Mountain Herbarium at Laramie, Wyo.

## GEOLOGY OF ROCK CREEK REGION

The geological formation [1] belongs to the lower part of the Montana group of the Cretaceous, characterized by sandstones and carbonaceous shales with local coal deposits. The depth of this deposit is said to average over 305 meters, and there is no evidence that Rock Creek has more than scratched the surface, so the soil of the region is of nearly uniform character. Sandstones beneath the coal contain various fossils of particular interest, because about 50 per cent belong to genera represented there to-day, as cottonwood (*Populus* spp.), alder (*Alnus* spp.), birch (*Betula* spp.), willow (*Salix* spp.), and others. The rather soft surface shales and sandstones are occasionally exposed, but more frequently are covered with a fine gravel wash, somewhat disguised by reason of the growth of short grasses and xerophytic shrubs. These remarks, of course, apply only to the land above the stream. Rock Creek, like all the streams in the Laramie Basin, flows through a valley varying in width and filled with alluvial deposits, in many places 50 to 60 feet deep. The character of the upland soils is also typical of a great deal of the Laramie Basin, so that from an agricultural standpoint the results of this study are applicable to a much larger region than that in which the actual observations were made.

## CLIMATE OF ROCK CREEK REGION

The climate of Rock Creek is essentially that of the Laramie Basin. Meteorological records kept at the State University at Laramie since 1891 show an average rainfall of about 10 inches and a mean annual temperature of about 40° F. The monthly averages for 15 years are given in Tables I and II.

TABLE I.—*Monthly means of precipitation (in inches) at Laramie, Wyo., 1891–1905*

| | | | | | |
|---|---|---|---|---|---|
| January | 0.23 | May | 1.47 | September | 0.92 |
| February | .34 | June | 1.24 | October | .79 |
| March | .83 | July | 1.40 | November | .22 |
| April | 1.14 | August | .99 | December | .33 |

TABLE II.—*Monthly means of temperature (°F.) at Laramie, Wyo., 1891–1905*

| | | | | | |
|---|---|---|---|---|---|
| January | 21.6 | May | 47.4 | September | 51.8 |
| February | 20.3 | June | 56.6 | October | 42.1 |
| March | 28.4 | July | 62.3 | November | 31.1 |
| April | 37.3 | August | 61.9 | December | 21.8 |

The higher temperatures are of short duration, and the maximum rarely reaches 90° F. All the nights are cool. The flora is thus, of necessity, composed of plants that have become adapted not only to the low average temperature and the aridity of the plains but also to the

[1] Darton, N. H., and Siebenthal, C. E. Geology and mineral resources of the Laramie Basin, Wyo. A preliminary report. U. S. Geol. Survey Bul. 364, 81 p., 2 pl. 1909.

short season; for although September often draws to a close before the first killing frost, the next June may be half gone before the cottonwoods along the streams have flaunted anything like full-grown leaves. Of course, grasses and bulbous and thick-rooted perennials have beautified the plains with flower or verdure long before the last frost is out of the meadow lands, or at least before the spring freshets have subsided enough to permit the growth of the meadow plants.

## PHYSIOGRAPHY OF ROCK CREEK REGION

Physiographically the Rock Creek ranch is divisible into four regions or units which for convenience may be designated and defined as follows: (1) The stream valley (Pl. XCVIII), practically synonymous to the flood plain of the creek and characterized by the natural meadows, willow thickets, swamps, and cottonwood timber; (2) the bench slope (Pl. XCIX; C, fig. 1), representing the sides of the stream valley; (3) the draws, or gullies (Pl. C, fig. 2), occurring on the bench proper and breaking through the bench slope at intervals; and (4) the bench land (Pl. CI, fig. 1), flat, short-grass, upland plains.

A knowledge is needed, of course, of the original flora of the flooded lands, in order to comprehend the changes in vegetation which are going to take place in some of the regions as the result of irrigation. Accordingly the immediately succeeding paragraphs are devoted to a description and to an analysis of this flora.

## PHYTOGEOGRAPHY OF ROCK CREEK REGION

The lists of plants under "Phytogeography of Rock Creek region" represent only those that contribute a present or later value toward the working out of the problem in hand. For additional species noted, mostly of interest only to the botanist, the reader is referred to the lists of minor plants on pages 757–758. In some instances plants here referred to under their generic name are given their specific designations later and in these instances will not be found in the supplementary lists.

### BENCH FLORA

#### CHARACTERISTIC BENCH-LAND PLANTS

*Agropyron* spp. (four species).
*Buchloe dactyloides* (Nutt.) Engelm.
*Oryzopsis hymenoides* (R. and S.) Ricker.
*Eurotia lanata* (Pursh) Moq.
*Potentilla effusa* Dougl.
*Astragalus* spp. (five species).
*Oxytropis monticola* Gray.
*Pentstemon angustifolius* Pursh.
*Chrysothamnus frigidus* Greene.
*Artemisia frigida* Willd.
*Lygodesmia juncea* Don.
*Tetradymia inermis* Nutt.

The ecologist will note plants in this list representative of well-known plant associations, such as the short-grass and the wheat-grass; the purpose of these lists, however, is not to classify the plants of a given physiographic unit but rather to treat such plants as a complex the limits of which are

determined purely by the bounds of the unit, as indicated above. Since these bounds are of more or less arbitrary definition in themselves, the disposition of plants within them is of like nature, though it may be said that, in general, the fullest development of a given species normally occurs within the unit in which it is placed.

Any one of the four wheat-grasses may be dominant or principal species—that is, it may make up the bulk of the vegetation over large areas of this unit (Pl. CII, fig. 2). The kind with which we are most concerned, however, reaches its best development in another complex.

Indian millet (*Oryzopsis hymenoides*), though of great importance from a nutritive standpoint,[1] is largely confined to loose, somewhat sandy soils; and therefore its possibilities for forage development are limited to regions of that nature.

Attention is called to the large number of legumes in this complex—five kinds. These will be augmented by five more, distributed among the remaining complexes. Legumes require rich soils of high lime content, the significance of which will develop later.

The remaining plants are nearly all xerophytes, species eminently fitted in one way or another to withstand the rigors of the environment in which they live. It is not surprising that such plants will take no part in an artificial transformation of this bench to meadow, where new conditions are suddenly introduced which are directly opposed to those to which these species have become so well adapted. Their disappearance in many cases means the loss of the most nutritious plants on the range.

CHARACTERISTIC DRAW, OR GULLY, PLANTS

*Bouteloua oligostachya* (Nutt.) Torr.
*Koeleria cristata* (L.) Pers.
*Stipa comata* Trin. and Rupr.
*Carex* spp. (two species).
*Zygadenus intermedius* Rydb.
*Delphinium Geyeri* Greene.
*Astragalus* spp. (two species).
*Lupinus parviflorus* Nutt.
*Antennaria parvifolia* Nutt.
*Artemisia cana* Pursh.
*Grindelia subalpina* Greene.

The bench lands are interrupted at irregular intervals by swales, or draws. These carry away the surplus surface water to the stream, so that those that drain a considerable area become gulches. Plate C, figure 2, gives a good average idea of this topographical feature. The chief difference, as compared to the bench proper, is the more constant water supply, which is somewhat greater and which lasts longer. It is not surprising, therefore, that over 69 per cent of its more characteristic plants (as given above) will persist (in some cases attain greater development) during the first season under irrigation.

Among those that will not be able to stand the new conditions are *Delphinium Geyeri* and *Zygadenus intermedius*, both a constant menace to

[1] Knight, H. G., Hepner, F. E., and Nelson, Aven. Wyoming forage plants and their chemical composition—Studies no. 3. Wyo. Agr. Exp. Sta. Bul. 76, 119 p., 50 fig. 1908.

stock because of poisons they contain. The latter is peculiar in its habitat relations. It appears in drier parts of meadows that have been established for several years, but does not seem to stand the sudden change to meadow conditions. This may be explained by the fact that it grows in the lower parts of the draw, where the change in moisture content becomes greatest. When it later invades the meadow, places frequently exist that more nearly conform to its usual habitat. At any rate, the new meadows are free from it for some time.

### CHARACTERISTIC BENCH-SLOPE PLANTS

*Agropyron Smithii* Rydb.
*Elymus condensatus* Prsl.
*Delphinium Menziesii* DC.
*Arabis hirsuta* Scop.
*Potentilla pennsylvanica* L., var. *strigosa* Pursh.
*Astragalus* spp. (three species).
*Solidago concinna* A. Nels.

The change from upland to lowland is very abrupt, as shown in Plates XCI and C, figure 1. The conditions are in many respects similar to those of the preceding region, except that the water supply is greater and even more constant. This permits the growth of various mesophytic herbs and shrubs (for lists of these see pp. 757–758.) The upper part of the slope is more or less like the bench, depending upon the local variation in the abruptness of the incline. Therefore the bench-slope plants can not be satisfactorily segregated from those of other units, since species characteristic to them find within the borders of the bench slope entirely suitable habitats.

*Agropyron Smithii* prefers a more constant water supply than its relatives, so it is placed here, though a complete list of the plants of either of the complexes previously discussed would contain it. *Elymus condensatus* likes a moist, sunny, well-drained situation; consequently it is out of the question as a meadow plant and is not of much value under any condition, being coarse and woody. *Delphinium Menziesii* no doubt is poisonous, but its numbers are ordinarily so limited that its interest lies chiefly in the fact that it is a relative of *Delphinium Geyeri*. *Arabis hirsuta* at times acts very much like a weed. One of the three vetches at home here is *Astragalus tenellus*, the only upland vetch in the new-meadow development.

### METHOD OF IRRIGATION USED

Before considering the transition itself—that is, the artificial transformation of upland to meadowland—the reader should know something of the mechanical means employed. Although the ranch owners have taken some pains to build large reservoirs, such as the one shown in Plate CI, figure 2, the actual distribution of the water over the land is accomplished in the crudest way imaginable. A ditch is built along what is obviously the highest ridge of a given area and provided with the

customary boxes where they seem to be needed. Very few laterals are used, and there is no secondary ditching. The ditch is opened at intervals and the water allowed to creep out over the land. Naturally it follows the path of least resistance. In order that it may reach some of the high places as well as all of the low, temporary ridges of earth are thrown up, which may perhaps be termed "dikelets," since they are too small and unstable to be called "dikes," but which serve the same purpose. Although, of course, this is irrigation in a broad sense, "controlled flooding" would be a term both more accurate and more appropriate for a method in which science plays so small a part. The remarkable thing about this is that the area so treated really receives a very even soaking; and if there are a few higher places that are not as wet as the land as a whole, so there are higher places in the natural meadow that are always relatively dry. After all, then, controlled flooding furnishes conditions almost analogous to those present in the natural meadow, an important point which, once attained, makes it possible to realize that these uplands, geologically the same as the valley they inclose, potentially are capable of the same vegetative results.

## THE TRANSITION

The first interest in this transition may be expressed by the question, "What happens to the upland plants?" The great bulk of them perish very soon. However, assuming that the water is turned on in the spring and allowed to remain until the soil is saturated and is thereafter replaced at intervals frequently enough to keep the ground wet (the common procedure), many of the plants may reach maturity. These readily fall into two classes: First, those of little economic value, either because of actual numerical or structural deficiency or because of lack of ability to cope successfully with the new conditions; and, second, those which flourish under the new conditions and often possess great economic value. The following represent the first class:

UPLAND PLANTS OF SOME IMPORTANCE FIRST SEASON IRRIGATED

*Oryzopis hymenoides* (R. and S.) Ricker.
*Stipa comata* Trin. and Rupr.
*Potentilla pennsylvanica* L., var. *strigosa* Pursh.
*Astragalus tenellus* Pursh.
*Lupinus parviflorus* Nutt.
*Pentstemon exilifolius* A. Nels.
*Chrysothamnus frigidus* Greene.
*Gaillardia aristata* Pursh.
*Solidago concinna* A. Nels.
*Tetradymia inermis* Nutt.

I have already spoken of the limitations of Indian millet. Not infrequently though, an area of upland will contain one to several spots, which with controlled irrigation could be much more profitably used for growing millet than rushes. The rushes are not nearly so nutritious, but, as will be shown later, will monopolize these sandy places under the controlled-

flooding system. With moderate moisture millet becomes even prolific and attains a height of about 60 cm.

*Stipa comata* is a good pasture grass, but the long awns may be fatal to stock because of the presence of tiny barbs. On the range the animals seek it either before the awns have developed or after they have fallen. Altogether, it is fortunate that it is able to survive only one season of meadow conditions.

The next plant that needs more than mention is vetch, the only one persisting of the 10 upland kinds listed. Its ability to persist makes it the connecting link between the upland vetches and those of the lowland which are to invade the developing meadow.

The lupins (Pl. C, fig. 2; CII, fig. 1), which persist a summer under controlled flooding, could probably be perpetuated indefinitely under controlled irrigation. However, since some species are strongly suspected of being poisonous to stock, at least during certain periods of their growth, their cultivation can not be advised, unless careful means are taken to ascertain this fact as regards the local species. These are numerous, and some are known to be not only harmless but of great value. Such is the case with an Idaho species which I have seen form, with the farmer's encouragement, a nearly pure stand on rather sandy bottom lands. This was cut just before the pods became dry, and when fed mixed with hay, both cattle and horses relished and thrived on it during the winter.

The next list represents the chief contribution of the upland flora to the growing meadow formation.

UPLAND PLANTS TENDING TO DOMINANCE THE FIRST SEASON IRRIGATED

*Agropyron albicans* R. and S.
*Agropyron dasystachyum* (Hook) Scribn.
*Agropyron molle* Rydb.
*Bouteloua oligostachya* (Nutt.) Torr.
*Koeleria cristata* (L.) Pers.
*Carex siccata* Dewey.
*Carex stenophylla* Wahl.
*Arabis hirsuta* Scop.
*Antennaria parvifolia* Nutt.
*Grindelia subalpina* Greene.

Nearly a dozen upland plants not only persist under controlled flooding but even flourish, at least when they happen to be established on a relatively high spot. With the advent of the first meadow plants (some of which are next listed) two opposing elements must be considered, one of which has had to adapt itself to an environment partially new.

MEADOW PLANTS APPEARING ON UPLAND THE FIRST SEASON IRRIGATED

*Hordeum jubatum* L.
*Sporobolus brevifolius* (Nutt.) Scribn.
*Juncus longistylis* Torr.
*Astragalus Bodinii* Sheld.
*Astragalus hypoglottis* L.
*Oxytropis deflexus* DC.
*Plantago eriopoda* Torr.

By the end of the first summer these typically meadow plants are almost certain to be represented, sometimes only by scattered individ-

uals, again completely appropriating areas left barren because of the death of upland plants that were unable to grow in the new environment.

These two elements furnish the foundation for two distinct lines of development which the upland may undergo in its transformation. The value of the respective components may be noted profitably now.

*Agropyron Smithii*, strangely enough, seems to be the only species that develops either vigorously or abundantly. There are at least two possible reasons for this. Attention has already been called to the fact that it prefers the slopes where the moisture content is greater and more uniform, so it may be the only species that is capable of using to advantage the increase in available moisture. Or it is not impossible that some of the other kinds, under the new conditions, become indistinguishable from the true *A. Smithii*, with which they not infrequently grow and from which they are separated by such characters as pubescent glumes and comparative awn development, characters which there is good reason to believe are easily modified by environmental factors.

*Koeleria cristata* and *Bouteloua oligostachya* and the two sedges noted are valuable, but the controlled-flooding method of irrigation caused their almost total disappearance by the second season.

*Arabis hirsuta* develops best, as will be evident when controlled irrigation is used, becoming one of the few native weeds.

*Antennaria parvifolia* is an everlasting nuisance on comparatively dry places. Plate CII, figure 1, shows its excessive development along a meadow edge. It readily succumbs to too much water, but unhappily the regular order of succession seems to mean that it will usually be replaced by *Pedicularis crenulata*, the weed that so frequently ruins meadows.

*Grindelia subalpina* reaches its greatest development the second season, as a weed.

*Hordeum jubatum* is not necessarily a meadow weed by any means, but since it occurs as such in this region it is included in this list of meadow plants. It is sometimes fed, but is dangerous because of the barbed awns. Like wheat-grass, interest in it has just begun.

*Sporobolus brevifolius* and *Plantago eriopoda* are of saline or subsaline habitat, and therefore their presence is significant. When an alkaline place appears, the invasion of this valuable [1] grass is the best thing that can happen; if it succeeds in establishing itself, it uses a spot that would be occupied by the plantain or later by one or both species of Sagittaria. (See "Minor plants," pp. 757–758.)

*Juncus longistylis* is destined to play an important part in the meadow development. Its nutritive value is rightly regarded as good, but it suffers by comparison of its analysis with that of wheat-grass.

[1] Knight, H. G., Hepner, F. E., and Nelson, Aven. Op. cit.

It is not surprising to find these meadow vetches appearing so early when the numerous upland species are recalled, a proof of the upland and lowland similarity as regards the soil and a further index to its character as pointed out in a preceding paragraph. *Astragalus bodinii* develops long, spreading stems which make it difficult to harvest, but its high nutritive value and the evident relish with which it is eaten overcome this drawback. Though its development may be arrested, it will ultimately form a large part of the hay crop.

The other species, *A. hypoglottis*, does not seem able to hold its own long where *A. Bodinii* is present, and the latter will eventually largely replace it.

*Oxytropis deflexus* belongs to a different habitat and is to be regarded here as a stray. It may be poisonous, but it rarely occurs in any abundance.

Such, then, is the condition of vegetation at the end of the first season. During the second season one of two possible lines of development become definitely established. Either the forming meadow enters upon what may be called the Agropyron phase or else the Hordeum phase. As to which phase appears depends on the following condition: It was observed that the ranch company sometimes ran short of water; and in such a case the newer areas were kept only moist, not wet like those regions longer established, for which the greater part of the available water was conserved. Granted that one or more of the wheat-grasses happened to be dominant over a given area the year before (as was very likely the case) a practically pure stand of this grass was the result (Pl. CV). The presence of an occasional tuft of *Hordeum jubatum* should be noted.

Before tracing through the development of this phase, the other possibility should be mentioned. As a curtailed water supply was essential to the dominance of *Agropyron* spp. at this time, so an abundance produced the Hordeum phase (Pl. CIII, figure 1). This phase is more strongly defined than the other because *Hordeum jubatum* is always the dominant plant. Its chief competitor is *Grindelia subalpina*, which is just as worthless and just as truly a weed.

Here is striking evidence of the aggressive character of a plant which because of its uselessness we call a "weed" and of the unobtrusive character of an indigenous and valuable grass. That is, the development of the Agropyron association is primarily dependent on its local dominance the preceding year. When such is not the case, the artificial condition described above tends to produce a mixed association characterized by one or all of those plants which largely composed the upland and the meadow elements, as discussed previously. Chief among these will be *Bouteloua oligostachya* and *Koeleria cristata*, the two sedges *Carex siccata* and *C. stenophylla*, *Antennaria parvifolia*, the meadow vetches

*Astragalus Bondinii* and *A. hypoglottis*, and *Juncus longistylus*. *Grindelia subalpina* and *Hordeum jubatum* are usually present in varying abundance but never attain dominance. This development should be contrasted with that of the Hordeum association. Here we have dependence only on the water factor whereas the Hordeum association was dependent not only on this but also primarily on its own dominance the preceding year. In the case of an abundant water supply, whether or not *Hordeum jubatum* was locally present, let alone dominant, the first season, it attained dominance the second. Attention has also been called to the stray plants of *H. jubatum* appearing either in the pure or mixed types of the Agropyron association, a further indication of its aggressiveness.

In the outline of the meadow activities during the second season no mention has been made of possible new emigrants from the lowlands. As some are destined to have a most important share in the further history of the evolutive upland, the following list is given:

MEADOW PLANTS APPEARING ON UPLAND SECOND SEASON IRRIGATED

*Deschampsia caespitosa* (L.) Beauv.
*Juncus balticus* Willd. var.
*Juncus bufonius* L.
*Rumex mexicanus* Meisn.
*Epilobium Drummondii* Haussk.
*Glaux maritima* L.
*Orthocarpus luteus* Nutt.
*Veronica peregrina* L.
*Gnaphalium palustre* Nutt.
*Rudbeckia hirta* L.

*Deschampsia caespitosa* is one of the best meadow grasses. It is frequently associated with *Calamagrostis canadensis* (Michx.) Beauv., which, strangely enough, seemed to be completely absent in the Rock River territory. It is highly probable, however, that it would bear the same relation as *D. caespitosa* toward the various factors involved in the present problem. It is highly nutritious,[1] material collected at this altitude containing 7.76 per cent of crude protein (water-free).

*Juncus balticus* ranks with *J. longistylus* in forage value. *J. bufonius* is a low diffuse annual.

*Rumex mexicanus* is always scattering in its distribution, but is usually a perceptible element in natural-meadow hay. It is probably to be regarded as a weed; it is coarse and certainly hard on sickle blades.

The rest are harmless herbs. *Orthocarpus luteus* under some conditions may become quite weedy in character. *Glaux maritima* furnishes by its presence another evidence of saline soil. It is as worthless for food as *Plantago eriopoda*.

A careful examination of either of the associations defined above would show the presence of at least some of these plants. They would be scattered through the dominant species in an entirely inconspicuous manner. In a large way the Agropyron phase would contain a suggestion of *Deschampsia caespitosa*, *Juncus longistylus*, and *Orthocarpus luteus*,

[1] Knight, H. G., Hepner, F. E., and Nelson, Aven. Op. cit.

and other herbs, and the Hordeum phase, *J. balticus*, *Rumex mexicanus*, *Grindelia subalpina*, and others mentioned. However, this does not mean that *D. caespitosa*, for instance, would be absent from the latter phase, but merely that it would occur more frequently in the former; and so with *J. balticus*, which would be less abundant, though not necessarily lacking, in the Agropyron association, and so on.

Before the end of the second growing season seeds of the following meadow plants will have germinated and taken root in the upland, so that by the middle of the next (the third) summer, they will have become a factor to be reckoned with. Therefore their consideration at this time is not out of place.

MEADOW PLANTS APPEARING ON UPLAND THIRD SEASON IRRIGATED

*Carex nebraskensis* Dewey.
*Carex Gayana* Desv.
*Carex lanuginosa* Michx.
*Carex marcida* Boott.
*Gratiola virginiana* L.
*Agoseris glauca* (Pursh) Steud.

*Carex nebraskensis* is one of the commonest and most valuable[1] of our sedges. It grows in clumps and reaches a good height. It is very similar to *C. variabilis*, which is perhaps even more frequent.

*Carex Gayana* is often the principal species of a given area. It is much less robust than the other sedges and does not grow as tall. Another drawback is that it has a tendency to mature early and turn yellow, losing much in substance before harvest. *C. lanuginosa*, next to *C. nebraskensis*, is probably the most important of the sedges. It is highly nutritious,[1] and its long narrow leaves and slender stems make it quite grasslike.

The remaining species are mainly of interest because of their comparatively early appearance. They belong to that large series of meadow plants which are present here, absent there, and are usually late in coming in. A large proportion of the swamp species belong to this type (p. 753). By the end of the second summer, then, there has been a considerable invasion of meadow plants.

Plate CIII, figure 2, clearly shows what happens during the third season. The reader will recall the presence of rush in the second-year condition of the Agropyron association. Here it is rapidly replacing the wheatgrass (stunted by too much water) and in the fall this phase were better called the Juncus-Carex, or rush-sedge phase. The next summer (the fourth) it will ordinarily reach its highest development. "Ordinarily," because this step in the program is no doubt dependent on the resumption of the usual controlled flooding, a method which, it will be remembered, was temporarily forsaken because of insufficient water. During my observation this resumption always took place, because by this time the upland had reached the stage where a hay crop was assured and good treatment justifiable. Owing to the relatively dry conditions, *J. longi-*

[1] Knight, H. G., Hepner, F. E., and Nelson, Aven. Op. cit.

*stylus* was the more abundant rush rather than *J. balticus*, which likes the wetter areas; similarly *Carex Gayana* and *C. marcida* outranked *C. nebraskensis*. *C. lanuginosa* was not present. The hay made from this association for the fourth season showed a marked increase in the percentage of *Deschampsia caespitosa*.

In the Hordeum phase the evolution is somewhat different and often less rapid than in the rush-sedge phase. Controlled flooding, the factor which, by drowning all competition, made possible the dominance of *Hordeum jubatum*, the next year weakened this same dominance. *Juncus balticus*, and even *Deschampsia caespitosa*, are much better suited to prolonged wettings. The result was that by the fourth season, the rush-sedge phase had been evolved here also, but with less definiteness unless complete destruction of the virulent weed, *H. jubatum*, was hastened in a novel and unexpected way. In some cases this annihilation was actually accomplished by the sudden appearance of the smut, *Ustilago hordei* (Pers.) Kell. Acres of the grass were often affected, it being practically impossible to find a single plant which did not have all or nearly all of its heads completely smutted. The coming of the new enemy, coupled with the already serious crowding of plants better suited to the wet conditions, could result in only one thing. The rush-sedge supplanted the Hordeum phase, just as the latter had supplanted the Agropyron phase, except that in this case its components were other species. The rush was *J. balticus*, the sedges were *Carex nebraskensis* and *C. lanuginosa*, and the proportion of *D. caespitosa* was often much greater. When the smut did not appear, at least another year was necessary for the drowning out of *H. jubatum*.

Thus, by the fourth year, or at least by the fifth, the upland normally reached the condition we have called the "rush-sedge phase", a condition characterized by the presence of plants belonging in large part to the rush or sedge groups or families. This phase is more stable than either of the earlier stages (the Agropyron or Hordeum); but it, too, soon changes.

The growing abundance of *Deschampsia caespitosa* has been mentioned; in fact, in some cases it came on so rapidly that it became established at the same time as the Juncus-Carex phase forming what might be called the Juncus-Carex-Deschampsia phase. This was particularly likely to be the case when the upland passed through the Hordeum stage At any rate, a year or two usually showed the condition illustrated in Plate CIV, figure 1. This appears to be a nearly pure stand of *D. caespitosa*; but in reality it contains a small percentage of rush, principally *J. balticus*, if its origin has been by way of the Hordeum phase, or of *J. longistylus* and sedge, if its development has been through the Agropyron phase.

At this time there is a tendency of certain plants to attain local dominance. Naturally among these are *Carex Gayana*, *C. nebraskensis*, and

*Astragalus Bodinii.* The species of Carex have a high water requirement and belong to the cycle resulting from the continuous use from the first of controlled flooding. The best development of the vetch was coincident to the Agropyron phase; otherwise its growth was limited to isolated, often more gravelly, elevated places of relative dryness. The exact conditions that produce these subphases, as they may be called, are not understood and represent only one of the many points which have yet to be fully worked out.

The Deschampsia phase is by far the most stable yet considered. Once established, controlled flooding seemed to satisfy its water requirement so exactly that its character fluctuated but little from year to year. The areas longest under water, however, gave evidence of two possible further changes; either a gradual increase in the abundance of rush (largely *Juncus balticus*) or a gradual increase in the number of kinds of plants. The former case meant the ushering in of the *Juncus balticus*, or wire-grass, phase the presence of which typifies swamp conditions and is the forerunner of the bog. Besides wire-grass, the hay harvest from this phase at Rock Creek contained some at least of the following plants:

SWAMP SPECIES TENDING TO INVADE WETTER PARTS OF MEADOW

*Calamagrostis hyperborea* Lange.
*Glyceria borealis* (Nash) A. Nels.
*Eleocharis palustris* (L.) R. and S.
*Scirpus microcarpus* Presl.
*Habenaria viridiflora* Cham.
*Rumex occidentalis* Wats.
*Ranunculus reptans* L.

Of these plants only two are ordinarily of sufficient importance to deserve comment. *Eleocharis palustris*, naturally an inhabitant of pond margins, finds in the wire-grass phase an environment to which it is well suited and it soon becomes an important factor. Its value as forage is fully as great as that of the rushes.[1] *Scripus microcarpus*, like the preceding, is indicative of marshy conditions. It is scattering in its distribution, but its presence is to be noted with satisfaction, as it is one of the few members of this phase that possess a considerable forage value.[1]

The other possibility, the gradual increase in the number of different species, is yet to be considered. This change is even slower than the other. For one thing, there is now a firmly established turf in which any plant finds it difficult to secure a foothold. Finally, however, now here, now there, some local variation in conditions or some factor which escapes our notice permits the invasion of many species, such as those listed under the heading "Meadow plants appearing at some later season" (p. 758). Only three of these are noteworthy: *Cicuta occidentalis* is a poisonous parsnip which for its best development needs the conditions that produce the wire-grass phase. *Pedicularis crenulata* is no doubt the worst weed of the natural meadows, but strangely enough does not seem to bother the made meadows until late. If it appears, the *Carex Gayana*

[1] Knight, H. G., Hepner, F. E., and Nelson, Aven. Op. cit.

subphase seems to be its most likely point of attack. *Carduus foliosus* is sometimes very troublesome. Fortunately it, too, is mostly confined to the natural meadow.

Gradually, then, the upland takes on the cosmopolitan character of the natural meadowland shown in Plate CIV, figure 2. In other words, those finer adjustments that involve plants which possess keener sensibility to moisture content and to slight soil variations are brought about by nature only after many years until finally there exists that marvelous complex of species growing in perfect equilibrium which is known as the natural meadow.

## ECONOMIC APPLICATION OF OBSERVATIONS

Such, then, is the story of the artificial production of a natural meadowland. One distinct type of vegetation completely supplanted another type of vegetation. This change was relatively gradual and not abrupt; it was readily divisible into periods, each period or stage being characterized by certain species which dominated that period, only to be recessive in the next; and lastly, the forage values of the various plants have been given.

These observations have proved that it is possible to control the physical stages of the evolutive upland. Therefore, if stockmen desire to augment their yield of natural hay, their procedure should be as follows:

First, a careful study of the upland area to be transformed should be made. It was shown that in the case of the Rock Creek project the upland and lowland were of the same geological formation and the soils of essentially the same character. Presumably this is important, but no study has been made of dissimilar regions. The principal plants should be noted, and if the area were large those parts which supported a good stand of wheat-grass or lupin (see above) or Indian millet should be mapped out, because on this knowledge would depend an intelligent use of the available water.

Second, a surveyed and modern system of irrigation should be established for use on the tract, so that not only controlled flooding but also controlled irrigation could be employed as occasion demanded. The latter method is essential for the development of the Agropyron phase, which was eliminated at Rock River, unless by chance the water supply was curtailed and conditions produced simulating those made possible by controlled irrigation. Briggs and Shantz[1] have shown that *Agropyron Smithii* has a water requirement of 1,076 units for every unit of dry weight produced, as compared with alfalfa with 831. With facilities for controlled irrigation the farmer should be able to furnish with a little practice the amount of water required to develop to the fullest the

[1] Briggs, L. J., and Shantz, H. L. Relative water requirement of plants. *In* Jour. Agr. Research, v. 3, no. 1, p. 1–63, pl. 1–7. 1914. Literature cited, p. 62–63.

latent possibilities of this native grain. The advisability of the preservation of this phase from year to year is a little doubtful. It might be successful, but it would probably never give the yield to the acre that the Deschampsia phase furnishes and is less nutritious, unless the later phase contains a large percentage of *Juncus balticus*. Besides, it is not improbable that after a year or two *Hordeum jubatum* would be able to maintain itself in such abundance that it would ruin the wheat-grass crop (Pl. CII, fig. 2). Then, too, controlled irrigation is more expensive than controlled flooding, so that the question of practicability enters. Altogether, the evidence seems to indicate that, whenever possible, the Agropyron phase should be encouraged for a year, or possibly two, in order to eliminate the Hordeum phase (the constant result of controlled flooding) and then should be allowed to pass into the Juncus-Carex or Juncus-Carex-Deschampsia phase, according to the natural tendency.

In starting the transformation all precautions should be taken to eliminate the worthless Hordeum phase, a stage which, once established, means from one to several seasons lost, with no advantage to the farmer. In case wheat-grass was not present on the upland, controlled irrigation tended to the development of a mixed association.

Considering the additional expense, it is thought probable that in most cases the Hordeum phase had better be tolerated, as the transition may be more rapid to the Deschampsia phase.

The rush-sedge phase (the next, the reader will recall, in the cycle of normal succession) should not be encouraged. It is doubtful whether there is any escaping its presence, although a uniform and constant water supply is probably the chief factor that tends to modify it into the Juncus-Carex-Deschampsia phase. Care must be taken, lest with too much water it revert to *Juncus balticus*. Whether evolved from the Agropyron or the Hordeum phase, none of its chief components possess the nutritive value of *Deschampsia caespitosa*. *Carex lanuginosa* ranks the highest, but it is likely to form a subphase of its own.

The two principal species of the Deschampsia phase represent extremes in forage value. *D. caespitosa* is one of the most nutritious meadow plants[1] and *Juncus balticus* is one of the least nutritious. Hence, the importance of maintaining *D. caespitosa* as the dominant plant of this phase. This accomplished, the farmer has the best natural meadow the region affords, which will average 1½ tons to the acre and will remain for years free from meadow weeds.

If too much water is used, this phase becomes replaced by *Juncus balticus*. The only species in this phase that in any way makes up for the loss of *Deschampsia caespitosa* is *Scirpus microcarpus*. If the farmer allows the Juncus phase to enter, he should consider that his meadow has reverted. The next step is the bog, which would mean the destruc-

[1] Knight, H. G., Hepner, F. E., and Nelson, Aven. Op. cit.

tion of the meadow. However, nature works just the other way. Aquatic plants tend to fill up the bogs (see p. 758); then come the species of the *J. balticus* phase. Besides being so much less nutritious than the *D. caespitosa*, the hay of this phase will not average a ton to the acre; often only half a ton.

Now compare the man-made natural meadow with the nature-made natural meadow. If the highest type of the former, the Deschampsia stage, be taken as the standard, it far excels the natural condition. This is because the latter is usually that mixed type to which the Deschampsia phase tends, perhaps always ultimately reaches. *Deschampsia caespitosa* may be the most conspicuous plant in a natural meadow, but it rarely, if ever, is truly dominant over any considerable area. It is more likely to contain plants which are either useless or actually harmful and must be regarded as meadow weeds. The yield of the natural meadow is seldom as high to the acre as that of the Deschampsia phase of the artificial. On the other hand, the rush-sedge and *Juncus balticus* phases often have their counterparts in the natural meadow, so that by producing these the farmer gains nothing more than an increased acreage. So long as the Deschampsia phase can be maintained, not only a greater quantity but also a better quality of natural meadow hay has been obtained.

## MEADOW HAY VERSUS ALFALFA AND GRAIN

In the spring of 1914 two tracts on the upland of this ranch, which so largely had been converted to meadow, were sown, one to alfalfa and the other to oats. The land was improperly prepared, and the distribution of water was uneven and poorly regulated. In spite of these obvious drawbacks, the two fields had produced by the end of the season the crops shown in Plate CV. It had been impossible to obtain definite data from the foreman of the ranch, but the yield of oats probably reached 30 bushels to the acre. Considering the way in which they were grown, this is a good yield. The illustration shows the excellent stand of alfalfa. A field on a neighboring ranch which was partly dry-farmed, there being water only during the fore part of the season, produced between 3 and 4 tons of alfalfa to the acre. If we recall the large number of native legumes the upland supported, we will not be surprised at the success of this legume, a plant notoriously fond of sweet soils rich in lime.

Plate CV, figure 1, shows the upland as it usually looks the first season under water, the strong development of *Antennaria parvifolia* being noteworthy. It is here that *Arabis hirsuta* flourished and became weedlike.

It must not be concluded that the artificial raising of natural hay does not pay. There are factors to be reckoned with which have not yet been considered. In the first place, it must be remembered that a natural meadow, once established, is fixed so long as the water supply holds out. On the other hand, alfalfa at this altitude needs reseeding every few

years, and oats often fail to mature. Besides, grain can not be grown indefinitely on the same land, even virgin land, without rotation. In the second place, the expenses of growing the cultivated crops are far greater than those of growing the uncultivated. The farmer who grows a natural meadow even by controlled irrigation as outlined above, ultimately will have less expense than the farmer who grows grain or alfalfa and continually has to repair ditches and regulate the water supply. Besides, he has not only been saved the initial cost of buying seed and of preparing a seed bed but he has also been able to utilize his entire upland, for all of it is potentially a meadowland.

These conclusions apply only to farming under the climatic conditions that exist at Rock Creek. At lower altitudes, where conditions are less rigorous and where cultivated crops well suited to the region have been long grown, this method of raising hay can not be too strongly condemned. It could be used with success only where hundreds of acres were available.

Even at Rock Creek on a smaller scale it would mean a criminal waste of land and water, as the ranch company is even now drawing so heavily on its reservoirs and on Rock Creek that during a dry season some of its meadows suffer. The owners have about reached the limit of increasing their hay yield by the growth of more natural meadows. Alfalfa gave much greater yields than the natural vegetation, and Briggs and Shantz have shown that alfalfa has a water requirement of only 831 units for every unit of dry weight produced. Further augmentation can come only by the raising of crops that require less water and give greater yields to the acre in return. The recent perfecting of grains and hays adapted to the high altitude and short season of the Laramie Plains will soon make this method of farming infeasible even under such conditions as those of Rock Creek.

## MINOR PLANTS

Below are lists of those plants occurring in the Rock Creek region which have not been mentioned in the body of this paper, being mostly of interest to botanists only.

### BENCH LAND

*Allium cernuum* Roth.
*Allium textile* Nels. and Macbr.
*Eriogonum ovalifolium* Nutt.
*Eriogonum flavum* Nutt.
*Paronychia sessilifolia* Nutt., var. *brevicuspis* A. Nels.
*Lesquerella condensata* A. Nels.
*Lesquerella montana* (Gray) Wats.
*Astragalus Drummondii* Dougl.
*Astragalus missouriensis* Nutt.
*Astragalus nitidus* Dougl.
*Astragalus Purshii* Dougl.
*Astragalus Shortianus* Nutt.
*Euphorbia montana* Engelm.
*Opuntia polyacantha* Haw.
*Cogswellia orientalis* Jones.
*Gilia pungens* (Torr.) Benth.
*Gilia spicata* Nutt.
*Phlox glabrata* (E. Nels.) Brand.
*Oreocarya flavoculata* A. Nels.
*Oreocarya thyrsiflora* Greene.
*Chrysopsis villosa* Nutt.
*Erigeron Eatonii* Gray.
*Sideranthus grindelioides* (Nutt.) Britton.
*Stenotus acaulis* Nutt.

DRAWS

*Calochortus Gunnisonii* Wats.
*Calochortus Watsonii* Jones.
*Eriogonum umbellatum* Torr., var. *intectum* A. Nels.
*Eriogonum campanulatum* Nutt.
*Arenaria congesta* Nutt.
*Astragalus pectinatus* Dougl.
*Astragalus succulentus* Rich.
*Viola Nuttallii* Pursh.
*Lithospermum angustifolium* Michx.
*Mertensia brevistyla* Wats.
*Senecio perplexus* A. Nels.

BENCH SLOPE

*Comandra pallida* A. DC.
*Fragaria ovalis* (Lehm.) Rydb., var. *glauca* (Wats.) A. Nels.
*Astragalus bisulcatus* (Hook.) Gray.
*Astragalus carolinianus* L.
*Thermopsis arenosa* A. Nels.
*Epilobium paniculatum* Nutt.
*Gilia pharnaceoides* Benth.
*Pentstemon alpinus* Torr.
*Castilleja linariaefolia* Benth.
*Pentstemon strictus* Benth.
*Achillea millefolium* L.
*Helianthus Nuttallii* T. and G.
*Lygodesmia grandiflora* T. and G.
*Amelanchier oreophila* A. Nels.
*Prunus demissa* (Nutt.) Dietr., var. *melanocarpa* A. Nels.
*Shepherdia argentea* Nutt.
*Cornus stolonifera* Michx.

STREAM VALLEY

*Salix Bebbiana* Sarg.
*Salix caudata* Muhl., var. *Watsonii* Bebb.
*Salix fluviatilis* Nutt.
*Salix fluviatilis* Nutt., var. *exigua* (Nutt.) Sarg.
*Salix Fendleriana* Anders.
*Populus fortissima* Nels. and Macbr.
*Betula fontinalis* Sarg.
*Alnus tenuifolia* Nutt.

AQUATIC PLANTS OF THE REGION

*Potamogeton perfoliatus* L.
*Lemna minor* L.
*Batrachium pantothrix* S. F. Gray, var.
*Callitriche palustris* L.
*Utricularia vulgaris* L.

WOODLAND SPECIES TENDING TO INVADE ADJACENT MEADOWS

*Carex aurea* Nutt.
*Juncus nevadensis* Wats.
*Arabis columbiana* Macoun.
*Cardamine Breweri* Wats.
*Oxytropis Lambertii* Pursh.
*Geranium Richardsonii* F. and M.
*Gentiana acuta*, var. *strictiflora* Rydb.
*Gentiana affinis* Griseb.
*Castilleja sulphurea* Rydb.
*Galium boreale* L.
*Campanula rotundifolia* L.
*Erigeron asper* Nutt.
*Senecio crocatus* Rydb.

MEADOW PLANTS APPEARING AT SOME LATER SEASON

*Equisetum hiemale* L.
*Triglochin maritima* L.
*Triglochin palustris* L.
*Alopecurus fulvus* Smith.
*Beckmannia erucaeformis* (L.) Host.
*Carex Douglasii* Boott.
*Carex valticola* Dewey.
*Juncus tenuis* Willd.
*Allium Nuttallii* Wats.
*Iris missouriensis* Nutt.
*Sisyrinchium idahoense* Bickn.
*Habenaria borealis* Cham.
*Erysimum cheiranthoides* L.
*Parnassia parviflora* DC.
*Argentina anserina* (L.) Rydb.
*Thermopsis divaricarpa* A. Nels.
*Vicia linearis* (Nutt.) Greene.
*Sidalcea neomexicana* Gray.
*Cicuta occidentalis* Greene.
*Zizia cordata* (Walt.) Koch.
*Dodecatheon pauciflorum* (Durand) Greene.
*Primula farinosa*, var. *incana* (Jones) Fernald.
*Pedicularis crenulata Benth.*
*Carduus foliosus* Hook.
*Crepis runcinata* (James) T. and G.

SUMMARY

The artificial formation of natural meadows is a gradual change divisible into several distinct periods or phases, each characterized by one or more particular species.

The relative permanence of these stages may be largely controlled by regulation of the water supply. By the same means any stage, in some measure, may be produced at will.

*Agropyron* spp. and *Deschampsia caespitosa* furnish the most valuable hay.

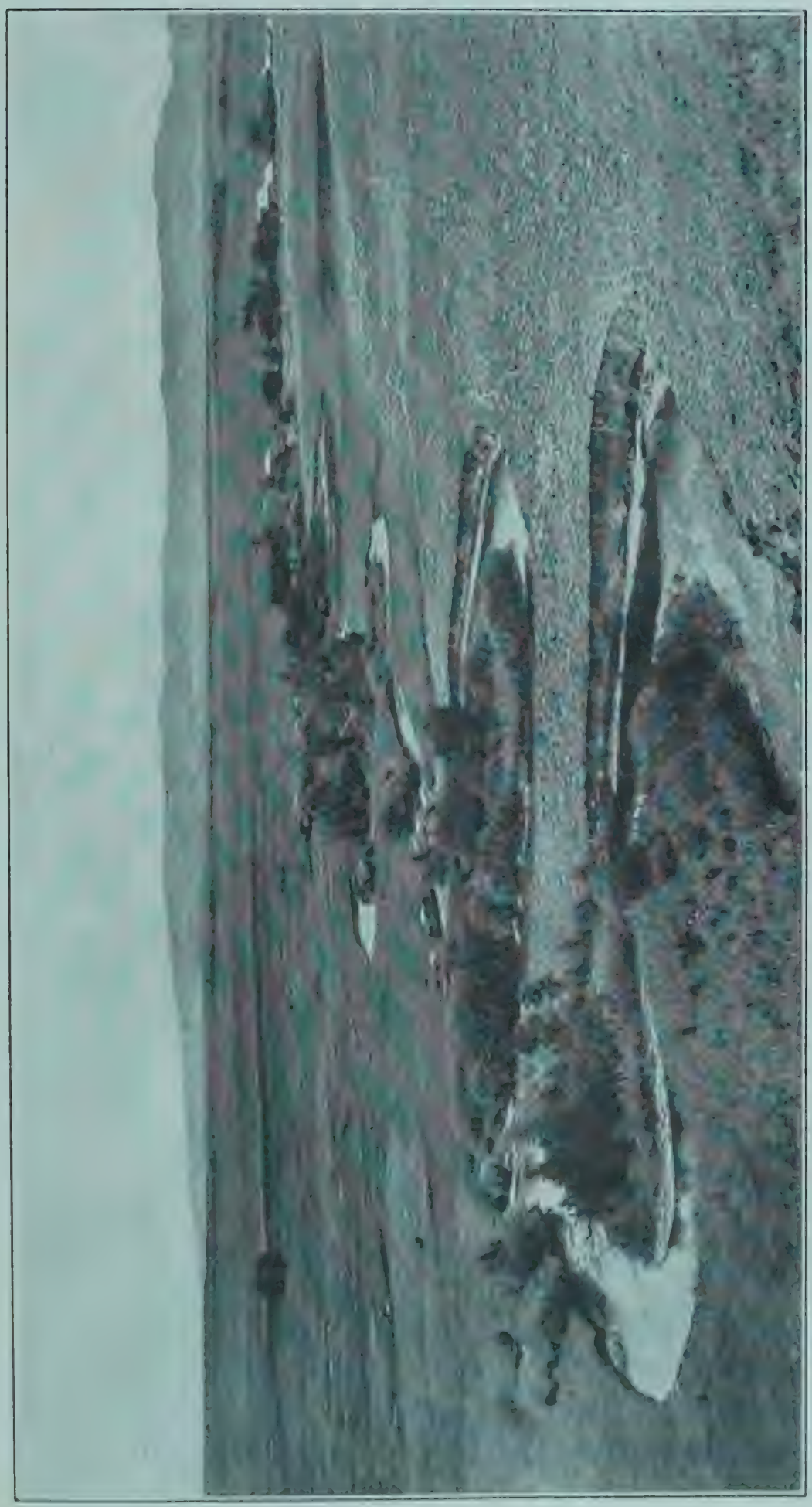
The yield of the natural meadowlands is generally smaller and less nutritious than the possible yield of the artificial. The latter meadows, however, tend ultimately to be composed of the same type of vegetation that characterizes the natural meadows; therefore it is important to watch and control their development.

This manner of hay raising is practicable at high altitudes where both land and water are abundant and domestic crops are uncertain. Where conditions are favorable for cultivated crops, the method would be wasteful and should be regarded as unsound farming.

With the growing scarcity of farming land and the development of crops suited to areas usually considered nonarable, the practical artificial formation of natural meadows will become limited to regions of even higher altitude and shorter season than those considered in this paper.

## PLATE XCVIII

Rock Creek Valley, near Rock River Station. Photographed by Dr. N. H. Darton, of the United States Geological Survey.



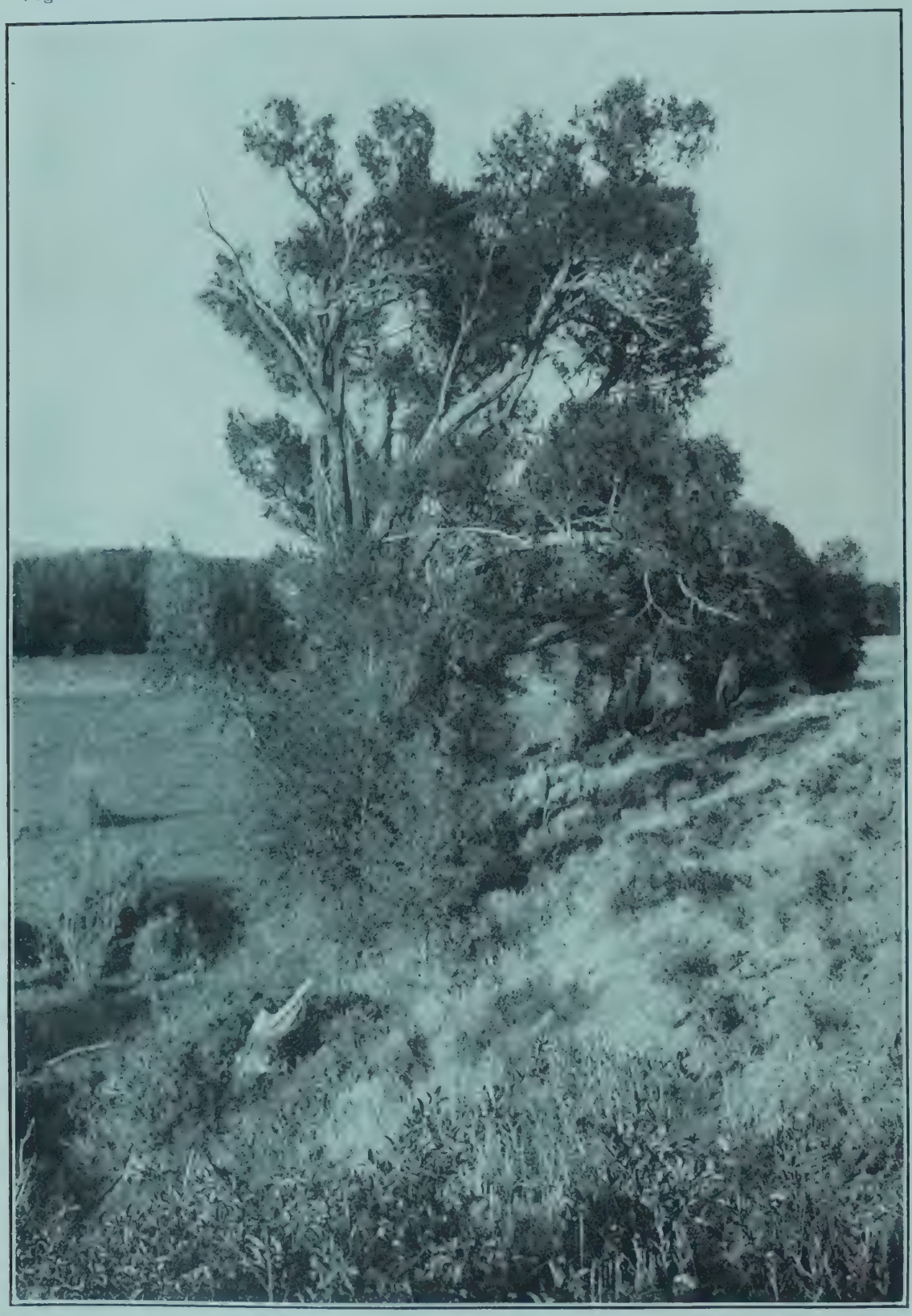


PLATE XCIX

A nearer view of the bench slope; the same tree shown in Plate C, figure 1.

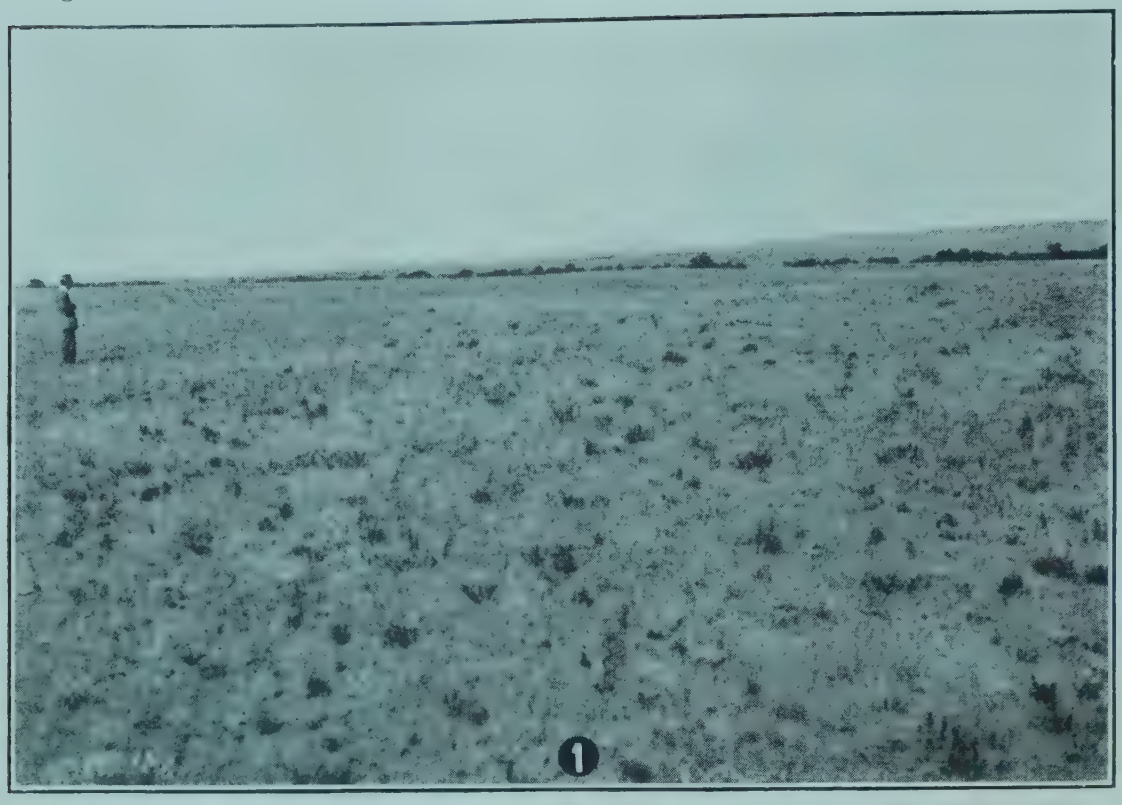
## PLATE C

Fig. 1.—Where upland and lowland meet. Notice the bench slope; described on page 745. For list of shrubs see "Minor plants, pages 757–758. The cottonwood tree is *Populus fortissima* Nels. and Macbr.

Fig. 2.—Characteristic draw; the stream valley beyond. Lupin, wheat-grass, white sage, and gaillardia in the foreground.

1

2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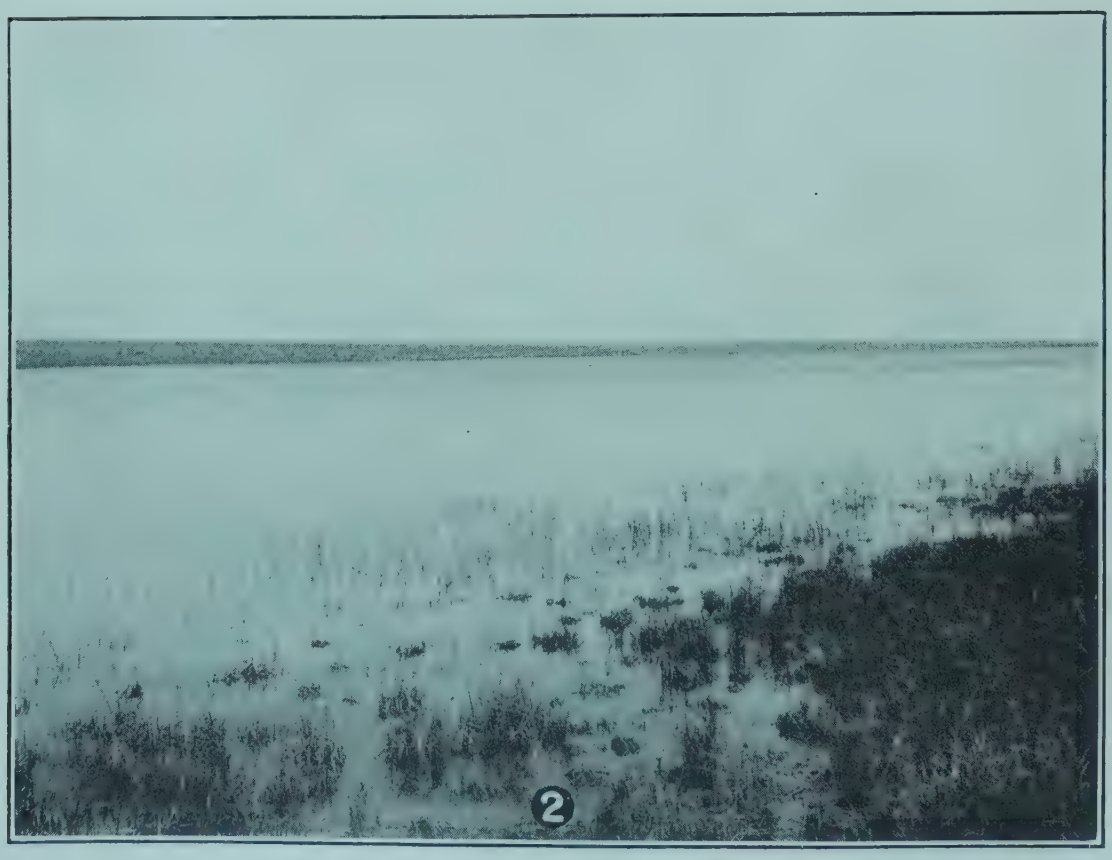
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## PLATE CI

Fig. 1.—The bench. The course of Rock Creek is indicated by the distant trees.
Fig. 2.—Part of a reservoir on the Rock Creek ranch.

## PLATE CII

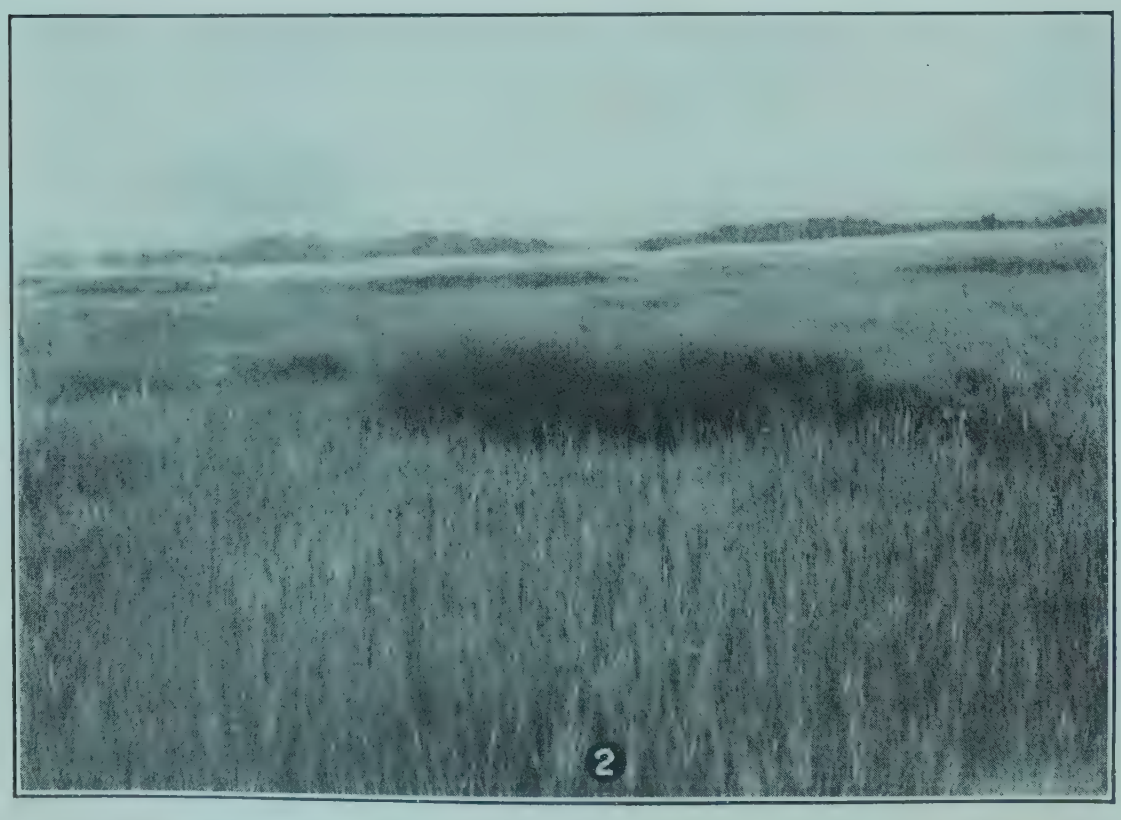
Fig. 1.—Lupin recessive and cat's-foot becoming dominant. Gay's sedge subphase in background.

Fig. 2.—Wheat-grass phase. Notice the occasional squirrel-tail grass.

1

2

1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## PLATE CIII

Fig. 1.—Squirrel-tail phase. A few grindelias in the foreground.
Fig. 2.—Rush-sedge phase (the darker areas) replacing wheat-grass phase.

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## PLATE CIV

Fig. 1.—Hair-grass phase.
Fig. 2.—Natural meadow. Note the cosmopolitan character of vegetation.

1

2

1

2

## PLATE CV

Fig. 1.—Field of oats on bench. Cat's-foot and other upland plants in foreground.

Fig. 2.—Alfalfa field one year after sowing. Cat's-foot and bench grasses in foreground.

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# AGRICULTURAL VALUE OF IMPERMEABLE SEEDS

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## INTRODUCTION

During the years 1909 to 1916, inclusive, many germination tests of lots of clover and alfalfa seed were made for the purpose of determining the agricultural value of the impermeable seeds. A smaller number of tests of winter vetch, okra, and other seeds were made for the same purpose.

Impermeable seeds, in the sense in which the term is used in this paper, are seeds whose coats are impermeable to water at temperatures favorable for germination. In the majority of plants which produce impermeable seeds this feature of the seed coat results from the peculiar character of its outer layer of cells, which may, in addition, be covered by a continuous cuticle.

Such seeds have been described by numerous investigators, including the present author, under the term "hard seeds." Guppy (9)[1] has, however, introduced a more appropriate term, "impermeable seeds," which will be used in the present paper. The term is relative, as impermeable seeds are capable of becoming permeable. While in the impermeable condition they remain very hard and dry, even when surrounded by water. When they become permeable, they absorb water readily, becoming soft and swollen. Naturally no seed can germinate while in the impermeable condition. In speaking of the germination of impermeable seeds, therefore, one means simply the germination of seeds which were impermeable at some previous time.

Many species of plants produce both impermeable seeds and seeds whose coats are readily permeable to water at one or more points. According to Guppy (9), these two types of seed can easily be distinguished by structural differences in certain plants (*Entada polystachya* and *Axyris amaranthoides*), but this is not true of any of the plants considered in this paper.

Verschaffelt (18) has investigated the relative permeability to both water and other liquids of different areas of the seed coats of a large

[1] Reference is made by number to "Literature cited," p. 796.

number of plants which produce impermeable seeds. The present author has found that many of the seeds of the species of plants considered in this paper are more readily permeable to water in the region of the chalaza than elsewhere.

Nobbe and Haenlein (16), Gola (8), Ewart (7), Rees (17), and Guppy (9) have given us a good idea of the distribution among the natural plant families of species which produce impermeable seeds. All agree upon the Leguminosae as far surpassing all other families in this respect. Many other families also contain species which produce impermeable seeds.

## OCCURRENCE OF IMPERMEABLE SEEDS IN CULTIVATED SPECIES

The peanut (*Arachis hypogaea*) excepted, probably all commercially important legumes cultivated in the United States produce a greater or less percentage of impermeable seeds. The percentage is small or fails entirely with spring vetch (*Vicia sativa* L.), some varieties of soybeans (*Soja max* (L.) Piper), kidney beans (*Phaseolus vulgaris* L.), Lima beans (*Phaseolus lunatus* L.), garden peas (*Pisum sativum* L.), and the newly introduced black bitter vetch (*Vicia ervilia* (L.) Willd.).

Table I shows the percentage of impermeable seeds in the commercial samples of a number of small-seeded legumes which have been tested by the Seed Laboratory during the six years 1904 to 1909.

TABLE I.—*Percentages of impermeable seeds in small-seeded legumes tested during the years 1904 to 1909*

| Kind of seed. | Number of lots tested. | Percentage of impermeable seeds. | | |
|---|---|---|---|---|
| | | Maximum. | Minimum. | Average. |
| Red clover (*Trifolium pratense* L.) | 1,642 | 46 | 0 | 9.61 |
| Alsike clover (*Trifolium hyridum* L.) | 304 | 40 | 0 | 10.16 |
| White clover (*Trifolium repense* L.) | 125 | 38 | 0 | 17.30 |
| White sweet clover (*Melilotus alba* Desv.) | 37 | 87 | 1.5 | 42.39 |
| Alfalfa (*Medicago sativa* L.) | 1,737 | 72 | 0 | 13.81 |
| Winter vetch (*Vicia villosa* Roth.) | 30 | 68 | 0. | 20.97 |
| Spring vetch (*Vicia sativa* L.) | 28 | 8 | 0 | 0.96 |
| Cowpea (*Vigna sinensis* (Torner) Savi) [1] | 37 | 60 | 0 | 3.55 |
| Toothed bur clover (*Medicago hispida denticulata* (Willd) Urban) | 6 | 85 | 9 | 48.08 |
| Spotted bur clover (*Medicago arbica* (L.) Huds.) [2] | 9 | 92 | 35 | 71.67 |
| Yellow-flowered sickle lucern (*Medicago sativa falcata* (L.) Döll) [1] | 5 | 86 | 35.5 | 49.72 |
| Yellow trefoil (*Medicago lupulina* L.) | 10 | 46 | 0 | 10.45 |

[1] Only two years' tests. [2] Only one year's tests.

Recent investigations by the author (10) show that the first four species of plants in Table I produce very much higher percentages of impermeable seeds than are indicated and that many of the seed coats become permeable to water during the operation of hulling the seeds.

Important nonleguminous plants which produce impermeable seeds are okra (*Hibiscus esculentus* L.) and hollyhock (*Althea rosea* (L.) Cav.), both

belonging to the Malvaceae, atriplex (*Atriplex* spp.), of the Chenopodiaceae, alfilaria (*Erodium cicutarium* (L.) L'Hér.), of the Geraniaceae, asparagus (*Asparagus officinalis* L.), of the Convallariaceae, morning-glory (*Ipomea purpurea* (L.) Lam.), of the Convolvulaceae, and canna (*Canna indica* L.), of the Cannaceae. The cherry-tomato (*Physalis pubescens* L.), of the Solanaceae, occasionally has some impermeable seeds.

## LONGEVITY OF IMPERMEABLE SEEDS

Although stating that some seeds with readily permeable coats may retain their vitality for many years in dry air, Ewart (7) was inclined to attribute extreme longevity of seeds in the soil exclusively to the impermeability of the seed coats. In contrast to Ewart's conclusion the work of Duvel,[1] Beal (1–5), and others indicates that great longevity of seeds even in moist soil may sometimes be the result of factors entirely independent of an impermeable seed coat. There is no doubt, however, that the possession of such a seed coat contributes to the length of life of the seed by decreasing or entirely preventing respiration and imbibition and, in general, by reducing the rapidity with which all physical and chemical changes take place within the seed. The seeds which Ewart (7) and Rees (17) have shown to retain their viability for 15 to 50 years or longer are almost exclusively seeds with impermeable seed coats. Beal, however, found the seeds of some typically permeable seeds (for example, *Brassica nigra*) viable after 30 years in the soil.

Becquerel (6) has reported the germination of impermeable seeds of three leguminous plants over 80 years old, and Ewart (7) mentions several plants as germinating from 5 to 80 per cent when over 50 years old.

According to Ewart, the curves of viability based on the germinating capacity of seeds of known age suggest 150 to 250 years as the probable extreme longevity of any known seed.

## EXPERIMENTAL WORK DURING 1909–1916

The major part of the work reported in this paper was done with the seeds of red clover, alsike clover, white clover, white sweet clover, alfalfa, hairy vetch (*Vicia villosa* Roth.), and okra. A small amount of work was done also with seeds of crimson clover (*Trifolium incarnatum* L.), black locust (*Robinia pseudacacia* L.), kidney bean (*Phaseolus vulgaris* L.), garden and field peas (*Pisum sativium* L.), cowpeas, and *Chamaecrista nicitans* L. Muench.

Nearly all chamber tests and greenhouse tests were made with two samples of 100 seeds each from each lot of seed tested. In some cases the number available was small and less than 200 seeds were used. A number of tests of okra were made with two lots of 50 seeds each. In the

[1] Unpublished data.

field from 250 to 500 seeds were used in testing each lot, 500 being tested in nearly every case.

In all of the germination tests which were conducted in the germinating chambers folded blue blotting paper free from soluble dye was used as germinating beds for the seeds of the clovers, alfalfa, black locust, and *Chamaecrista nicitans*, and folded canton flannel for the seeds of the other kinds of plants. In the green house tests both sand and steam-sterilized potting soil were used.

## VIABILITY OF IMPERMEABLE SEEDS

In May and June, 1914, 128 lots of seed from 1 to 5 years old were tested for germination, and the viability of the seeds remaining impermeable after six days was determined and compared with the viability of the seeds which softened in the first six days of the test.[1] Table II summarizes the results.

TABLE II.—*Viability of impermeable seeds from 1 to 5 years old*

| Kind and age of seed. | Number of lots. | Average percentage of— | | | |
|---|---|---|---|---|---|
| | | Germination. | Impermeable seeds. | Viability of impermeable seeds. | Viability of seeds which softened in six days. |
| Red clover: | | | | | |
| 5 years | 21 | 34 | 59 | 99 | 83 |
| 4 years | 5 | 43 | 43 | 96 | 75 |
| 3 years | 9 | 34 | 58 | 99 | 81 |
| 2 years | 11 | 39 | 60 | 99 | 98 |
| 1 year | 8 | 26 | 74 | 100 | 99 |
| Alsike clover: | | | | | |
| 3 years | 4 | 15 | 81 | 100 | 79 |
| 2 years | 6 | 7 | 90 | 100 | 70 |
| 1 year | 5 | 6 | 90 | 100 | 60 |
| White clover: | | | | | |
| 3 years | 1 | 8 | 82 | 95 | 44 |
| 2 years | 6 | 8 | 90 | 100 | 80 |
| 1 year | 4 | 12 | 87 | 100 | 92 |
| Sweet clover: | | | | | |
| 4 years | 2 | 1.5 | 98 | 100 | 75 |
| 2 years | 12 | 4 | 95 | 98 | 80 |
| 1 year | 3 | 10 | 89 | 100 | 91 |
| Alfalfa: | | | | | |
| 3 years | 6 | 71 | 25 | 100 | 95 |
| 2 years | 5 | 69 | 28 | 100 | 96 |
| 1 year | 7 | 67 | 32 | 100 | 99 |
| Hairy vetch: | | | | | |
| 5 years | 1 | 57 | 8 | 100 | 61 |
| 1 year | 3 | 59 | 17 | 100 | 71 |
| Crimson clover: 2 years | 2 | 65 | 26 | 100 | 88 |
| Okra: 3 years | 5 | 9 | 87 | 92 | 69 |
| *Chamaecrista nicitans:* | | | | | |
| 4 years | 1 | 36 | 43 | 85 | 63 |
| *Robinia pseudacacia:* | | | | | |
| At least 5 years | 1 | 25 | 54 | 95 | 54 |

[1] To determine the viability of the impermeable seeds, the seed coats of 20 seeds from each lot (or all seeds remaining impermeable after six days if not more than 20) were cut with a knife, and these seeds with cut seed coats were then subjected to germination conditions for seven days.

Over 90 per cent of the impermeable seeds were viable in every case, except the lot of seed of *Chamaecrista nicitans*. In most cases 100 per cent were viable. The average percentage of viability of the impermeable seeds was invariably greater than of the seeds which softened within six days. The difference ranged from 1 per cent to over 50 per cent—the latter with 3-year-old white-clover seed—and in general increased with the age of the seed.

RATE OF SOFTENING OF IMPERMEABLE SEEDS WHEN KEPT IN WET BLOTTERS

Table III shows the average rates of softening of seeds which had remained impermeable after 10 days in wet blotters. These seeds were kept in wet blotters for three years.

TABLE III.—*Rate of softening of impermeable seeds when kept in wet blotters*

| Kind of seed. | Description.[1] | No. of lots. | Average percentage of seeds impermeable after 10 days. | Average percentage of impermeable seeds as shown in preceding column which softened in time indicated. | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1 month. | 1 year. | 2 years. | 3 years. |
| Red clover | Hand-gathered | 20 | 86 | 8 | 30 | 44 | 55 |
| Do | Commercial | 6 | 11 | 9 | 27 | 45 | 55 |
| Alsike clover | Hand-gathered | 2 | 91 | 9 | 52 | 63 | 66 |
| Do | Commercial | 5 | 14 | 7 | 21 | 28 | 28 |
| White clover | do | 5 | 30 | 3 | 13 | 23 | 30 |
| Sweet clover | Hand-gathered | 2 | 97 | 5 | 27 | 35 | 44 |
| Do | Commercial | 4 | 24 | 8 | 21 | 29 | 37 |
| Alfalfa | Hand-gathered | 10 | 70 | 46 | 97 | 99 | 100 |
| Do | Commercial | 8 | 20 | 10 | 70 | 90 | 95 |
| Hairy vetch | Hand-gathered | 2 | [a] 65 | 13 | 85 | 97 | 98 |
| Crimson clover | do | 2 | 45 | 42 | 93 | 100 | ..... |
| Do | Commercial | 5 | 12 | 50 | 100 | ..... | ..... |
| Okra | do | 5 | [a] 56 | 34 | 91 | 96 | 98 |

[1] All hand-gathered lots of seed were gathered and hulled by hand a few days before the beginning of the tests. Commercial lots were of uncertain age, probably in most cases a little less than 1 year old.
[a] After 15 days.

1. Less than 10 per cent of the seeds of red clover, alsike clover, white clover, and sweet clover which remained impermeable after 10 days softened in one month; and from about one-third to a little over one-half of them softened in three years.

2. Nearly all of the impermeable seeds of alfalfa, hairy vetch, okra, and crimson clover which remained after 10 days softened in one year in wet blotters, but a very few of all except crimson clover remained impermeable after three years. There is a marked contrast in this respect between these species of plants and those named in the preceding paragraph.

3. The impermeable seeds in hand-gathered lots of alsike-clover seed and alfalfa seed softened much more rapidly than the impermeable seeds

in commercial lots of seed of the same plants. There was but little difference in this respect between the impermeable seeds of hand-gathered lots and commercial lots of the other species of plants.

Almost all of the seeds of each kind of plant which softened at any time during the three years germinated and produced vigorous seedlings.

Besides seeds of the species given in Table II a few impermeable seeds of kidney bean, garden pea, and cowpea were included in the tests. All of the beans and peas softened and germinated within three months, and all of the cowpeas within eight months.

### INFLUENCE OF MATURITY ON THE RATE OF SOFTENING OF IMPERMEABLE RED-CLOVER AND ALSIKE-CLOVER SEEDS IN WET BLOTTERS

The lots of hand-gathered seed included under Table III were thoroughly mature and dry in the heads before being removed from the plants. Figure 1 shows graphically the comparative rates of softening of such well-matured impermeable red-clover seed and of impermeable red-clover seed of two other degrees of maturity.

Seven of the eight lots of slightly immature seed were gathered at the same time and from the same cultivated rows of plants as were seven of the eight lots of mature seed, the former being taken from heads which were slightly green and succulent, the latter from black, dry heads. The three lots of light, immature seed used in the comparison were separated from three of the lots of slightly immature seed by a gravity blowing machine. Only seeds of good appearance, though frequently of small size in the immature lots, were used in the tests.

The average percentages of the mature seeds, the slightly immature seeds, and the more immature seeds which remained impermeable after 10 days in wet blotters and from which the rates of softening were calculated were respectively 84, 72, and 27.

Of the impermeable seeds from lots of light, immature seed, 78 per cent softened in one month and 100 per cent in 13 months. In contrast to this, only 5 per cent of the impermeable seeds from lots of well-matured seed softened in one month and 44 per cent of them remained impermeable after three years.

The differences in the rate of softening of impermeable seeds from lots of mature and immature seeds of alsike clover were similar to those shown for red-clover seed. No other species of plants were investigated for rate of softening.

Hiltner (11) has shown that the percentage of impermeable seeds and the rate at which they soften when placed under conditions favorable to imbibition may in some cases be greatly altered by previous drying. Although only seeds which seemed to be thoroughly dry were used in these experiments, it is possible that artificial desiccation of the less mature lots would have increased the percentages of impermeable seeds and de-

creased the rate at which they softened. As will be shown later, however (p. 775), moderate heating of thoroughly air-dried seeds of these plants has little or no effect under ordinary conditions.

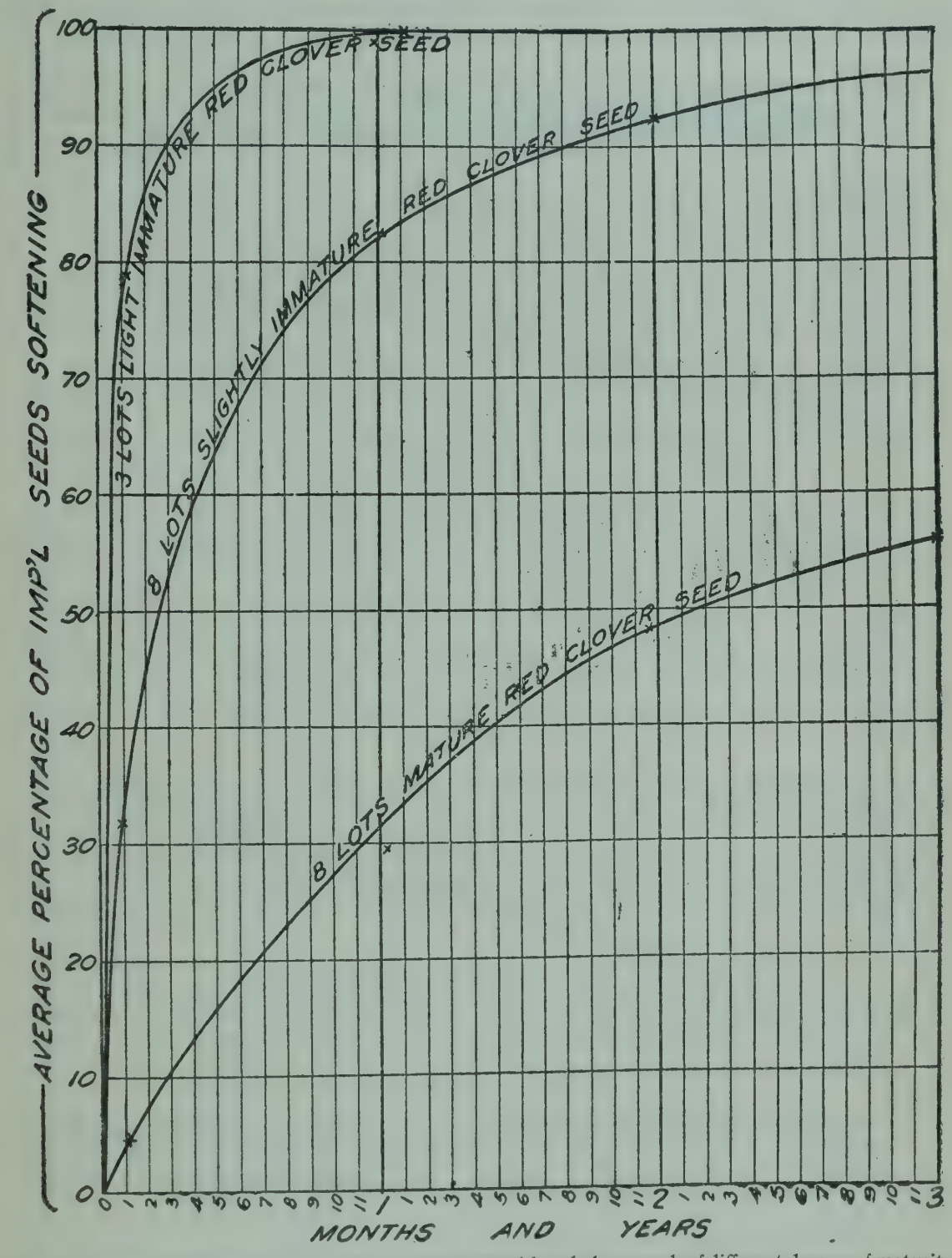
AVERAGE PERCENTAGE OF IMP'L SEEDS SOFTENING
100
90
80
70
60
50
40
30
20
10
0
3 LOTS LIGHT IMMATURE RED CLOVER SEED
8 LOTS SLIGHTLY IMMATURE RED CLOVER SEED
8 LOTS MATURE RED CLOVER SEED
0 1 2 3 4 5 6 7 8 9 10 11 1 1 2 3 4 5 6 7 8 9 10 11 2 1 2 3 4 5 6 7 8 9 10 11 3
MONTHS AND YEARS

FIG. 1.—Curves showing the rate of softening of impermeable red-clover seeds of different degrees of maturity.

## ESTIMATION OF THE GERMINABILITY OF IMPERMEABLE SEEDS

It is evident from the preceding discussion that it is impossible to estimate in advance what proportion of the impermeable seeds of a given lot will germinate under ordinary germination conditions in any given length of time. At one time Nobbe (14) proposed that one-third

of the seeds of red clover remaining impermeable after 10 days be reckoned as capable of germinating in one year. He later tested in distilled water 66 lots of red-clover seed from various sources, using 1,000 seeds of each lot. From 2.4 to 90 per cent of the seeds of the different lots which remained impermeable after 10 days softened in

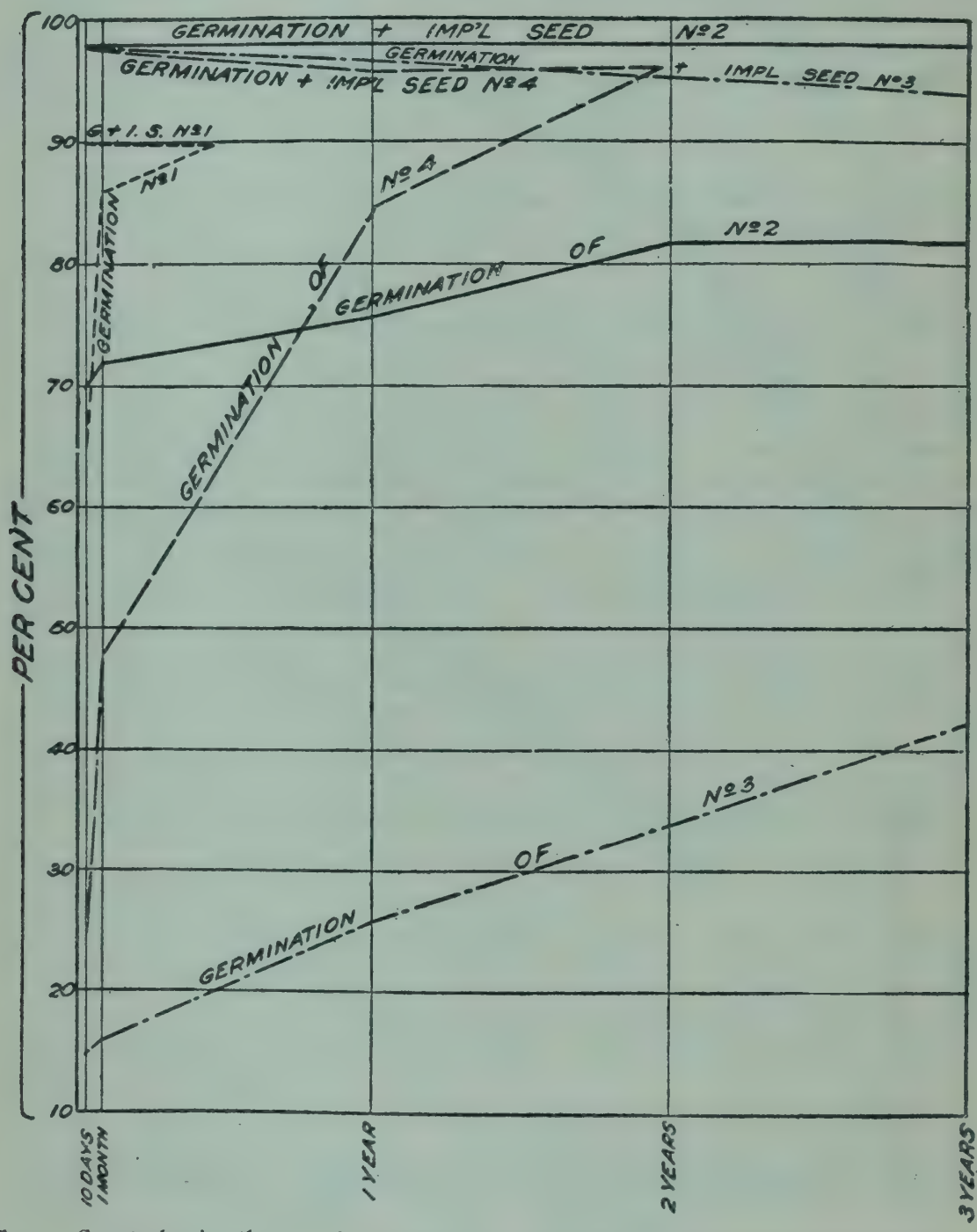


FIG. 2.—Curves showing the rate of softening and of germination of impermeable red-clover seeds of different lots.

distilled water in one year. On the basis of these results Nobbe (15) abandoned his previous position as untenable.

Different lots of seeds of any one of the species of plants included in Table III illustrate differences in the rate of softening of the impermeable seeds which are comparable to those reported by Nobbe. Figure 2

shows graphically the differences in this respect between four lots of red-clover seed. Lot 2 was commercial seed, while the other lots were seed which had been gathered and hulled by hand. While lot 3 was thoroughly dry when gathered, lots 1 and 4 were gathered when the heads were slightly green.

The lower of each pair of lines indicates the progress of germination of one lot of seed, the upper line the sum of percentages of germination and of impermeable seeds, and the space between the two lines of the pair the percentage of seeds which were impermeable at any given time.

1. All of the seeds of lots 1 and 4 softened and practically all germinated in, respectively, six months and two years. Yet only about 40 per cent of the impermeable seeds of lots 2 and 3 softened and germinated in three years.

2. Since the percentages of seeds of lots 1 and 4 which remained impermeable during the first 10 days were about the same as of lots 2 and 3, it is evident that the original percentage of impermeable seeds bears no relation to the rate at which these will soften or germinate.

3. It should be emphasized that the differences in maturity of the different lots of seed were not noticeable in the appearance of the seeds and offered, therefore, no basis for estimating the percentages of impermeable seeds which the different lots contained or the rates at which these impermeable seeds would soften.

### RATE AT WHICH IMPERMEABLE SEEDS BECOME PERMEABLE WHEN STORED IN MANILA ENVELOPES

Hand-gathered, hand-hulled seeds were tested for germination and impermeable seeds after being stored in manila envelopes for different lengths of time. The first test of each lot was begun a few days after the seeds were harvested, and the last test in some cases more than 4½ years later.

Table IV shows the calculated average percentages of the originally impermeable seeds in some of these lots of seed which became permeable in one month, one year, two years, three years, and four years. Loss of permeability during storage is indicated by the minus (−) sign.

1. The impermeable seeds of red clover, alsike clover, white clover, and sweet clover became permeable very slowly in dry storage. The red-clover seed changed more rapidly than the other kinds of clover seed, but less than one-half of the impermeable seeds of this species became permeable in four years.

2. The percentages of impermeable seeds in lots of alsike-clover, white-clover, and sweet-clover seed gathered in 1912 increased slightly during the first year and then remained about constant during the second year. This initial increase is probably due to the seed's being tested the first time before it was thoroughly dry. A similar increase occurred in a few lots of red-clover and alfalfa seed.

TABLE IV.—*Rate at which impermeable seeds became permeable when stored in manila envelopes*

| Kind of seed. | Year in which grown. | Number of lots. | Average percentage of impermeable seeds when gathered. | Calculated average percentage of the impermeable seeds as shown in preceding column which became permeable in time indicated. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 month. | 1 year. | 2 years. | 3 years. | 4 years. |
| Red clover | 1909 | 12 | 89 | 0 | ..... | ..... | 33 | 35 |
| | 1910 | 5 | 81 | ..... | 14 | 41 | ..... | 48 |
| | 1911 | 9 | 86 | ..... | 22 | ..... | 34 | ..... |
| Alsike clover | 1911 | 4 | 85 | ..... | 9 | ..... | 6 | ..... |
| | 1912 | 6 | 86 | −2 | ..... | − 3 | ..... | ..... |
| White clover | 1912 | 6 | 88 | ..... | − 5 | − 2 | ..... | ..... |
| Sweet clover | 1912 | 12 | 85 | ..... | −12 | −12 | ..... | ..... |
| Alfalfa | 1911 | 6 | 80 | ..... | 24 | 70 | ..... | ..... |
| | 1912 | 4 | 60 | 2 | ..... | 55 | ..... | ..... |
| Hairy vetch | 1909 | 1 | 73 | ..... | ..... | ..... | 90 | 95 |
| | 1913 | [a] 1 | 60 | ..... | 82 | ..... | ..... | ..... |

[a] This lot of hairy-vetch seed was grown by the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, at Chico, Cal.

3. Impermeable alfalfa and hairy-vetch seed [1] became permeable more rapidly than impermeable clover seed, 82 per cent of one lot of hairy-vetch seed becoming permeable in one year.

Besides the kinds of seed given in Table IV, five lots of okra seed gathered in 1911 were tested when fresh and six months, two years, and three years later. When fresh, all seeds softened and an average of 98 per cent germinated. Six months later only 23 per cent germinated and 71 per cent were impermeable. During the following two and one-half years very little change in permeability occurred.

It should be added that there was a slight decrease in the viability of the red-clover seed during the third and fourth years, and a large decrease in the viability of the vetch seed during the fourth year.

## VARIATION IN THE RATE AT WHICH IMPERMEABLE SEEDS OF A SINGLE SPECIES BECOME PERMEABLE

The impermeable seeds in some of the lots included in Table IV became permeable in dry storage much more rapidly than those of other lots of the same kinds of plants. In fact, the percentage of the impermeable seeds in different lots of red-clover seed which became permeable in four years varied from about 15 to about 80. This variation is further emphasized by the results of tests conducted in the fall and winter of 1914–15 and in September and December, 1915, using

[1] The vetch seeds in the lots here considered were thoroughly dry and black when first tested. Vetch seed, while it remains green in color and has a high water content, contains but a small percentage of impermeable seed or none. This percentage increases in storage for a time before any decrease takes place.

exclusively seeds grown in 1914.[1] Table V shows the average percentages of the viable seeds which were impermeable in the two tests and the calculated average and maximum percentages of the seeds, impermeable when freshly gathered, which became permeable in the interval of about one year between the two tests.

TABLE V.—*Change in permeability of clover and alfalfa seeds during the first year after harvesting*

| Kind of seed. | Manner of hulling. | Number of samples. | Average percentage of viable seeds which were impermeable. | | Calculated percentages of the seeds, impermeable when fresh, which became permeable in about 1 year. | |
|---|---|---|---|---|---|---|
| | | | Fresh. | 1 year old. | Average. | Maximum. |
| Red clover | Hand | 220 | 92 | 87 | 6 | 52 |
| Do | Machine | 207 | 17 | 14 | 19 | [1] 40 |
| Alsike clover | Hand | 12 | 91 | 91 | 0 | ..... |
| Do | Machine | 37 | 18 | 16 | 10 | [1] 26 |
| White clover | Hand | 8 | 98 | 90 | 8 | 55 |
| Do | Machine | 5 | 34 | 28 | 18 | [1] 40 |
| Sweet clover | Hand | 6 | 98 | 98 | 0 | ..... |
| Alfalfa | Machine | 2 | 32 | 7 | 60 | 87 |

[1] These calculations are based only on lots of which 30 per cent or more were impermeable when tested the first time.

The hand-hulled lots contained very large percentages and the machine-hulled lots comparatively small percentages of impermeable seeds. With few exceptions in the case of single lots of seed, the impermeable seeds in the hand-hulled lots became permeable more slowly than those in the machine-hulled lots.

The average results for all lots showed that not more than 8 per cent of the impermeable seeds in hand-hulled lots of the different kinds of clover seed had become permeable during the interval between the two tests; yet over 50 per cent of the impermeable seeds in one lot each of hand-hulled red-clover seed and hand-hulled white-clover seed became permeable.

The case of the hand-hulled white-clover seed is especially interesting. Eight lots averaged 98 per cent of impermeable seeds when fresh. Only 1 per cent of the impermeable seeds in seven of these lots became permeable in a little over a year. Of the impermeable seeds in the other lot 36 per cent became permeable in two months, 45 per cent in three months, and 55 per cent in 14 months. Nothing in the appearance of the different lots of seed either of white clover or of the other kinds indicated that any differences would be found in the rate at which the impermeable seeds became permeable.

[1] The results of the special investigation on impermeable clover seed conducted in the fall and winter of 1914–15 have been published elsewhere (10).

It should be added that no change could be detected in the viability of the seeds during the interval between the two tests except in case of the machine-hulled lots of red-clover seed and of alfalfa seed. With these there was a very slight decrease in viability.

### INFLUENCE OF MATURITY ON THE RATE AT WHICH IMPERMEABLE RED-CLOVER SEEDS BECOME PERMEABLE IN MANILA ENVELOPES

Four lots of red-clover seed were gathered in July, 1910. This was the earliest seed of a good grade that could be obtained from the plants in question. Four other lots of seed were gathered from the same cultivated rows of plants in October, 1910.

The average percentages of impermeable seeds in the lots of seed gathered in July and in October were, respectively, 71 and 82. When again tested for germination two years later, the average percentages of impermeable seeds were 35 and 62. In other words, one-half of the impermeable seeds in the less mature lots which were gathered in July and one-fourth of those in the more mature lots which were gathered in October became permeable in two years. There was practically no change in viability during the two years.

### COMPARATIVE RATES AT WHICH IMPERMEABLE SEEDS BECOME PERMEABLE IN WET BLOTTERS AND IN DRY STORAGE

A comparison of Tables III and IV shows that impermeable seeds become permeable more rapidly when kept under germination conditions than when stored dry. The difference in rates varies widely among different lots of the same species.

Between 20 and 40 per cent more of the majority of the lots of hand-hulled red-clover seed remained impermeable after four years in dry storage than after four years in wet blotters. With a few lots of red-clover seed, however, the differences were less than 5 per cent, and with a few other lots the differences were between 50 and 60 per cent.

Figure 3 represents graphically the changes in the percentage of impermeable seeds of typical lots of red clover, alsike clover, sweet clover, and alfalfa seed when kept in wet blotters and when stored dry in manila envelopes for various periods.

1. With each species the percentage of impermeable seeds decreased more rapidly in wet blotters than in dry storage.

2. The percentage of impermeable seeds decreased more rapidly during the first year than during succeeding years both in wet blotters and in dry storage.

### PRODUCTION OF SEEDLINGS IN SOIL BY IMPERMEABLE LEGUMINOUS SEEDS

Comparative tests were made in germinating chambers, in a greenhouse, and in soil outdoors, using lots of seed with varying percentages of impermeable seeds. The results of these tests indicate that, with rare

exceptions, very few of the impermeable seeds of the different kinds of clover, except crimson clover, will produce seedlings in the soil even in three months at temperatures such as prevail in late spring or in summer.

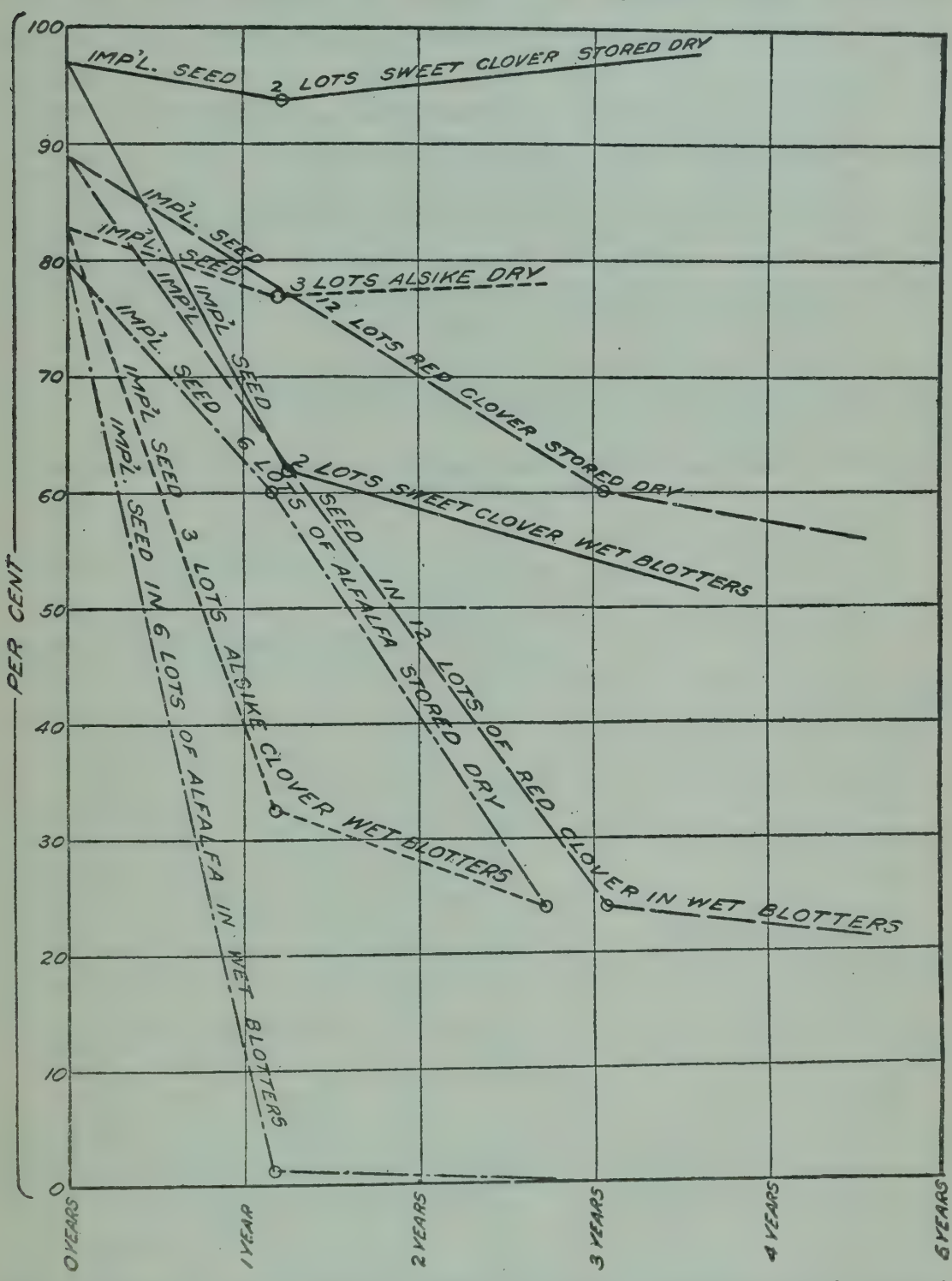


FIG. 3.—Curves showing the changes in the permeability of seeds in wet blotters and in dry storage for various periods.

The case of alfalfa, crimson clover, and the larger-seeded species is different. It was calculated that varying percentages of the impermeable seeds of alfalfa, hairy vetch, Canada field pea, cowpea, and okra produced seedlings in a few days or weeks both in a moderately warm

greenhouse and in greenhouse flats in which tests were conducted outdoors in warm weather.

In one experiment it was calculated that 16 per cent of the impermeable red-clover seeds, 5 per cent of the impermeable white-clover and sweet-clover seeds, and 38 per cent of the impermeable alfalfa seeds produced seedlings in greenhouse flats in three months. In the same time 5, 2, 3, and 8 per cent, respectively, germinated in a germinating chamber at room temperature, the rest remaining impermeable.

On June 7, 1909, seeds remaining impermeable during germination tests in germinating chambers were sowed in soil in large greenhouse flats, which were then set outdoors in the warm space between two greenhouses. The soil was kept watered, and observations were continued until November 30, 1909. During the latter half of November the temperature fell below freezing several times, but the last few days of the test were warm. The results of the tests are summarized in Table VI.

TABLE VI.—*Production of seedlings by leguminous seeds which remained impermeable during germination tests*

| Kind of seed and test No. | Number of seeds used. | Percentage of seeds which produced seedlings in— | | | |
|---|---|---|---|---|---|
| | | 1 month. | 2 months. | 3 months. | 6 months. |
| Red clover: | | | | | |
| 58593 | 110 | 5 | 7 | 8 | 8 |
| 83583 | 109 | 12 | 16 | 17 | 28 |
| 83843 | 76 | 3 | 5 | 5 | 5 |
| 85272 | 87 | 5 | 7 | 7 | 9 |
| 85341 | 43 | 5 | 5 | 5 | 5 |
| 85371 | 30 | 17 | 23 | 23 | 37 |
| 85454 | 25 | 8 | 8 | 12 | 16 |
| Average | ..... | 6 | 10 | 11 | 14 |
| Sweet clover: | | | | | |
| 78539 | 195 | 3 | 4 | 4 | [a] 14 |
| Alfalfa: | | | | | |
| 62874 | 12 | 75 | 92 | 92 | 92 |
| 78479 | 75 | 53 | 57 | 57 | 59 |
| 85276 | 30 | 80 | 90 | 90 | 90 |
| Average | ..... | 69 | 80 | 80 | 80 |
| Hairy vetch: | | | | | |
| 78326 | 24 | 38 | 38 | 38 | 38 |

[a] During the first 5 months of the experiment 4 per cent were produced; the remaining 10 per cent during the last few days of the experiment, following the freezing of the soil.

1. Only 14 per cent of the impermeable sweet-clover seeds and an average of 14 per cent of the impermeable red-clover seeds produced seedlings in six months.

2. An average of 69 per cent of the impermeable alfalfa seeds produced seedlings in one month, and an average of 80 per cent in two months, after which only one new seedling appeared in the next four months.

3. Thirty-eight per cent of the one lot of impermeable hairy-vetch seeds produced seedlings in one month, after which no new seedlings appeared.

4. It is worthy of notice that after four months, during which no new sweet-clover seedlings appeared, 10 per cent of the seeds used produced seedlings in a few days following the cold weather in November. This is particularly interesting in the light of subsequent results.

## EFFECT OF DIFFERENT CONDITIONS UPON THE GERMINATION OF IMPERMEABLE SEEDS IN SOIL

It has been shown that some seeds which would be reported as impermeable according to the chamber tests will produce seedlings in a comparatively short time in the soil. Experiments were conducted to determine the effects of separate factors.

Comparative tests showed that neither moistening the blotters which were used for germinating bed with strong aqueous soil extracts nor the alternate wetting and drying of the seeds at frequent intervals affects the rate of softening of impermeable clover seeds.

Neither the depth of planting nor the firmness of the soil nor the conservation of surface moisture by shading affected the precentage of seedling production by impermeable clover and alfalfa seeds in greenhouse tests, except when the seeds were planted over ¾ inch deep. Fewer seedlings reached the surface of the soil from seeds planted 1 inch deep than from seeds planted ¼ to ¾ inch deep.

On the other hand, certain factors influenced the softening of impermeable seeds and would probably affect the production of seedlings in the soil. These factors will be considered in the following sections.

## EFFECT OF HIGH TEMPERATURES UPON THE GERMINATION OF IMPERMEABLE CLOVER AND ALFALFA SEEDS

DRY HEATING.—Storing seeds for 6 months in a dry atmosphere at 45° C. slightly increased the subsequent germination of previously impermeable alfalfa seeds, but had no effect upon impermeable seeds of red clover or sweet clover. These results differ from results obtained by Hiltner (11). This author found that drying red-clover seed for eight days at 35° slightly increased both the percentage which remained hard after a 10-day germination test and the percentage which softened but did not germinate.

Heating at 50° C. for 21 hours had no effect upon the softening or germination of impermeable seeds of red clover or sweet clover when later subjected to a germination test.

HEATING IN WET BLOTTERS.—Seeds which remained hard after a 6-day germination test at 24° C. were subjected in the wet blotters to a temperature of 36° for the following seven days, during which time duplicate lots remained at 24°. Table VII shows the average percentages which germinated, and which softened but did not germinate.

TABLE VII.—*Softening and germination of impermeable clover and alfalfa seeds at 24 and at 36° C.*

| Kind of seed. | Number of lots. | Number of seeds tested at— | | Average percentage of germination at— | | Average percentage which softened but did not germinate at— | |
|---|---|---|---|---|---|---|---|
| | | 24° C. | 36° C. | 24° C. | 36° C. | 24° C. | 36° C. |
| Red clover | 60 | 3,220 | 3,227 | 0.48 | 1.53 | 0.09 | 0.34 |
| Alsike clover | 15 | 1,089 | 1,068 | 1.07 | 6.06 | .15 | .99 |
| White clover | 11 | 826 | 820 | .41 | .51 | 0 | 1.34 |
| Sweet clover | 17 | 1,604 | 1,607 | .94 | .94 | .06 | .45 |
| Alfalfa | 18 | 519 | 516 | 6.31 | 10.14 | 0 | .22 |
| Crimson clover | 2 | 48 | 56 | 1.92 | 18.27 | 0 | .96 |

1. A larger percentage of the seeds softened at 36° than at 24°. C. The differences were small with red-clover, white-clover, and sweet-clover seed, somewhat larger with alsike-clover and alfalfa seed, and over 16 per cent with crimson-clover seed.

2. With the exception of white-clover seed at 36° C. nearly all the seeds which softened germinated. However, a somewhat larger proportion of the seeds which softened failed to germinate at 36° than at 24°.

In view of the very slight effect of heating at 36° C. for seven days in wet blotters, it hardly seems possible that soaking clover seeds over night in water at 34° before planting can bring about the germination of impermeable seeds as suggested recently by Müller (13).

### EFFECT OF FREEZING TEMPERATURES ON THE GERMINATION OF IMPERMEABLE LEGUMINOUS SEEDS

One instance has already been mentioned in which impermeable sweet clover seeds previously lying dormant in the soil produced seedlings after a few days of freezing weather. (See Table VI.) A series of experiments was begun late in December, 1909, to test further the effect of freezing temperatures on the subsequent germination of impermeable leguminous seeds. The seeds used had lain in water without softening for 1½ to 5 months previous to the beginning of the experiment.

Different lots of the seeds were tested in a germinating chamber at about 20° C., in very moist soil in drinking glasses which were covered with black paper to exclude the light, and in soil in greenhouse flats. All of the seeds were subjected to freezing temperatures either before or during the germination tests, as follows:

The seeds which were tested in a germinating chamber at 20° C. were previously subjected, either dry or in small vials of water, to a temperature of about −10° C. One lot of each sample was given this treatment for 9 days and another lot during two periods of 9 days and 16 days, respectively, with an intervening period of a few hours in the laboratory at ordinary room temperature. As one exposure to this temperature had practically the same effect as two such exposures, only the average results of the tests of the two lots are herein considered.

TABLE VIII.—*Germination of impermeable leguminous seeds with and without freezing*

| Kind of seed. | Test No. | Germination, impermeable seeds, and dead seeds. | Check test in chamber. | Tested in chamber after freezing in dry condition. | Tested in chamber after freezing in water. | Tested in soil on window ledge; frozen during test. | Tested in soil in greenhouse flats; frozen during test. |
|---|---|---|---|---|---|---|---|
| Red clover | 85262 | Percentage of germination. | 6 | 8 | 26 | 21 | ..... |
| | | Percentage of impermeable seeds. | 93 | 92 | 72 | 65 | ..... |
| | | Percentage of dead seeds. | 1 | 0 | 2 | 14 | ..... |
| | 85272 | Percentage of germination. | 5 | 13 | 9 | 32 | 38 |
| | | Percentage of impermeable seeds. | 94 | 85 | 85 | 57 | ..... |
| | | Percentage of dead seeds. | 1 | 2 | 6 | 11 | ..... |
| Alsike clover | 84087 | Percentage of germination. | 12 | 14 | 10 | 17 | 50 |
| | | Percentage of impermeable seeds. | 87 | 82 | 80 | 72 | ..... |
| | | Percentage of dead seeds. | 1 | 4 | 10 | 11 | ..... |
| White clover | 85192 | Percentage of germination. | 3 | 10 | 11 | 10 | 30 |
| | | Percentage of impermeable seeds. | 96 | 89 | 88 | 81 | ..... |
| | | Percentage of dead seeds. | 1 | 1 | 1 | 9 | ..... |
| Sweet clover | 78539 | Percentage of germination. | 2 | 1 | 1 | 4 | 74 |
| | | Percentage of impermeable seeds. | 97 | 99 | 98 | 78 | ..... |
| | | Percentage of dead seeds. | 1 | 0 | 1 | 18 | ..... |
| Alfalfa | 78479 | Percentage of germination. | 4 | 20 | 14 | 6 | 56 |
| | | Percentage of impermeable seeds. | 95 | 80 | 84 | 84 | ..... |
| | | Percentage of dead seeds. | 1 | 0 | 2 | 10 | ..... |
| Black locust | ..... | Percentage of germination. | 1 | 14 | 7 | 2 | 8 |
| | | Percentage of impermeable seeds. | 99 | 84 | 90 | 96 | ..... |
| | | Percentage of dead seeds. | 0 | 2 | 3 | 2 | ..... |

The seeds in the drinking glasses were so placed that they could be examined through the glass. The drinking glasses were kept on a shaded window ledge outside the laboratory during two periods of 10 days and 14 days, respectively. Each of these periods included several cold days during which the soil became frozen clear to the bottoms of the glasses. During an intervening period of 9 days and again after the second period on the window ledge they were kept in the laboratory at ordinary room temperature.

The greenhouse flats were kept outdoors during the entire experiment. The soil in them was alternately frozen and thawed at intervals during the first two or three months.

Check tests were made in a germinating chamber at about 20° C. without previous treatment of the seeds.

All of the tests were continued until March 24, 1910, and those in the greenhouse flats until May 7, 1910.

Table VIII shows the percentages of the seeds which germinated or produced seedlings in the different tests, the percentages which remained impermeable, and the percentages which softened but did not germinate in all of the tests except those which were conducted in greenhouse flats.

1. Subjection to a freezing temperature previous to the germination test slightly increased the percentages of the impermeable red-clover, white-clover, alfalfa, and black-locust seeds which germinated. In some cases, especially when the seeds had been frozen in water, this treatment increased also the percentages which softened but did not germinate. This latter effect is partly the result of the fact that a part of the seeds which softened after the first period of freezing were killed by the second freezing.

The impermeable sweet-clover seeds were wholly unaffected by this treatment.

2. The effect of freezing the impermeable seeds in soil in drinking glasses was similar to but greater than the effect of subjecting them to a freezing temperature previous to a germination test in a germinating chamber. Nearly all of the seeds which softened following each period upon the window ledge softened during the first few succeeding days in the laboratory.

3. The percentages of the impermeable seeds of the clovers and alfalfa which produced seedlings in the greenhouse flats with frequent freezing and thawing were much greater than the percentages which germinated in the germinating chamber or in the drinking glasses. This was particularly noticeable with the sweet-clover and alfalfa seeds. Nearly all of the seedlings appeared during warm days immediately following freezing weather.

Only 8 per cent of the impermeable black-locust seeds produced seedlings, and these few seedlings appeared after settled warm weather had begun late in March.

EFFECT OF ALTERNATIONS OF TEMPERATURE ON THE GERMINATION OF IMPERMEABLE CLOVER AND ALFALFA SEEDS

Seeds remaining impermeable after from 4 months to over 12 months in wet blotters were kept in a chamber at room temperature for 49 days. The seeds were then kept for 50 days in chambers which, during a large part of the time, were heated daily to about 30° C. and then allowed to cool slowly to room temperature. Finally the seeds were again kept in chambers at room temperature for 51 days. Table IX summarizes the germination records for these three successive periods.

TABLE IX.—*Germination of impermeable clover and alfalfa seeds during successive periods of similar length with different temperature conditions*

| Kind of seed. | Number of impermeable seeds used. | Approximate percentages of germination during— | | |
|---|---|---|---|---|
| | | 49 days at room temperature. | 50 days with frequent heating to 30° C. | 51 days at room temperature. |
| Red clover | 2,382 | 3 | 7 | 1 |
| Alsike clover | 118 | 0 | 2 | 1 |
| White clover | 270 | 1 | 2 | 1 |
| Sweet clover | 637 | 2 | 1 | 1 |
| Alfalfa | 230 | 25 | 21 | 7 |

The use of the alternating temperatures increased very slightly the germination of red-clover, alsike-clover, and white-clover seed, but did not influence the germination of sweet-clover and alfalfa seed. In no case did more than 11 per cent of the impermeable clover seeds germinate in the five months included in the three periods of observation.

Clover seeds which remained impermeable after various lengths of time in wet blotters were tested for germination at 1° C. in an ice box averaging about 10°, at 20°, at 30°, and with daily alternations between each two of these temperatures. When alternations of temperature were used, the seeds were kept in the chamber at the warmer temperature for about seven hours of each day and in the chamber at the cooler temperature the remainder of the day. In each test a succession of several temperature conditions was used, each condition being maintained for several days.

Figure 4 shows graphically the results of a series of tests of red-clover seeds from a single original lot which remained impermeable after four days' soaking in water. During these tests many seeds softened and remained for several days after softening at a temperature too cold for their germination. These softened seeds, as well as those which softened at warmer temperatures, invariably germinated later if subjected to a temperature favorable for germination. In order to emphasize the effects of the different temperatures, figure 4 shows only the rates of softening of the seeds without regard to their immediate germination.

During the first few days of the test from 5 to 7 per cent of the seeds softened under each temperature condition, showing that not all the easily permeable seeds had been removed by the previous soaking. After the first seven days the rate of softening varied according to the temperature conditions of the different tests.

1. When the alternation of temperatures from the ice box to 30° C. was used after a period of incubation in the ice box the seed softened rapidly for a few days, but the rate of softening diminished greatly within a week and soon fell off almost entirely.

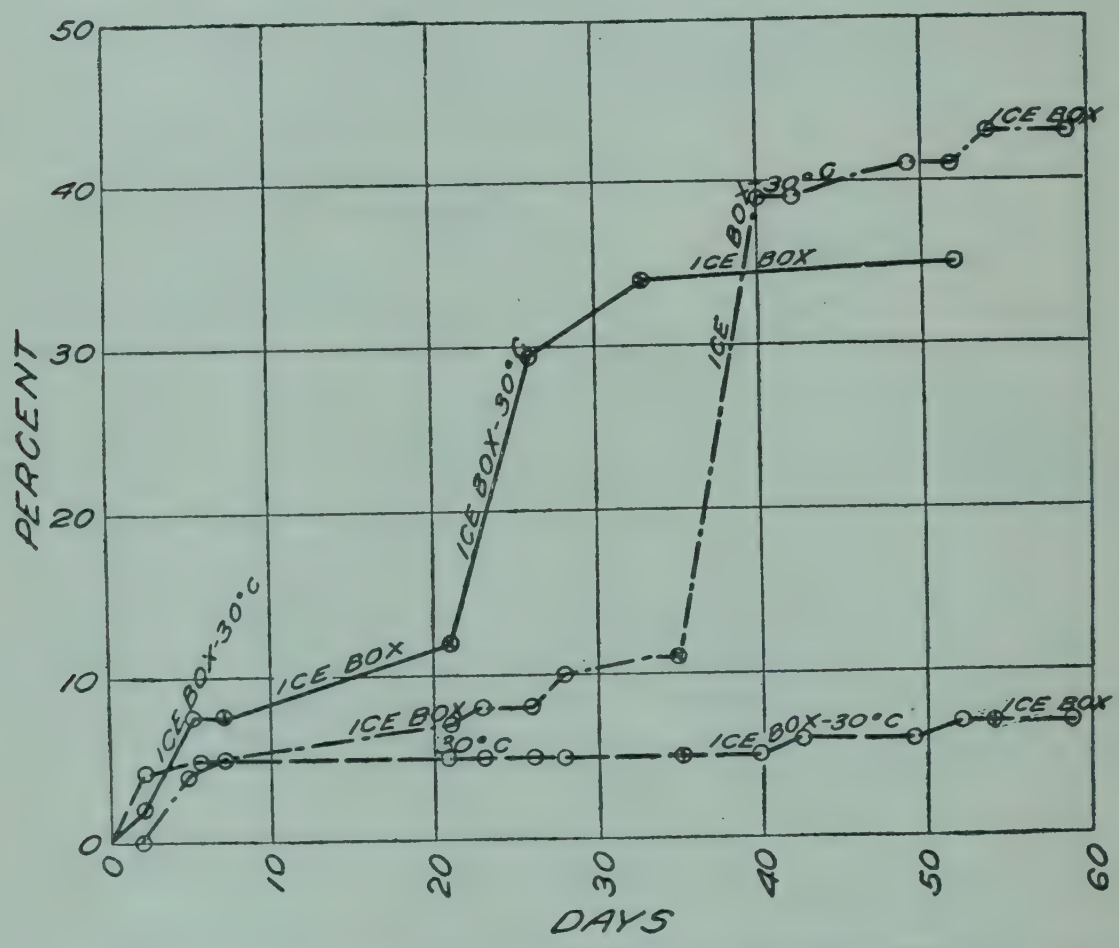


FIG. 4.—Curves showing the rate of softening of impermeable red-clover seeds under different temperature conditions.

2. While 32 per cent of the seeds softened when the alternation of temperatures was used after 35 days in the ice box, only 22 per cent softened with the same alternation after 14 days in the ice box.

3. When the alternation of temperatures followed 35 days at 30°, only 2 per cent softened in 19 days.

Results similar to those just outlined have been obtained with red-clover, alsike-clover, white-clover, and sweet-clover seeds which had previously remained impermeable in wet blotters for from two years to five years. The alternation from 1° to 30° C. has been as effective as the alternation from 10° to 30°. The alternation from 1° or 10° to 20° has had somewhat less effect. In any case the effectiveness of an alternation

has always depended upon a previous incubation of the seeds at a cool temperature. Usually the longer the previous period of cool incubation the greater has been the effect of the alternation. Alternations from 1° to 10° or from 20° to 30° had little or no effect.

In some cases more than 90 per cent of seeds which had previously remained impermeable in wet blotters for several years have softened and germinated in a few weeks with a favorable succession of constant cool temperatures and alternations of temperature.

The work outlined in this section is of special interest in connection with the following sections. The results here presented would lead one to expect that impermeable clover seeds would lie in the soil without change during either steady cold weather or constantly warm weather and in the fall, when an alternation of warm days and cool nights follows the hot summer months, but that many of them would germinate and produce seedlings at the beginning of the growing season in the spring when a similar alternation follows months of cold winter weather. These results also show that subjection to freezing temperatures is not necessary in order to prepare the majority of impermeable clover seeds for rapid germination. A temperature of about 10° C. does quite as well as 1°, and either of these temperatures is much more effective under favorable conditions than freezing temperatures under conditions which are less favorable. (See p. 776–778 and Table VIII.)

### GERMINATION OF SEEDS AFTER PASSING THE WINTER ON OR UNDER THE PARENT PLANTS IN THE FIELD

Seeds which had passed one winter on or under the parent plants in the field were gathered the following spring after warm weather had begun. The germinating capacity of these seeds and the percentages which were impermeable were determined and compared with the germinating capacity and percentage of impermeable seeds of lots of seed which were gathered from the same stands of plants the preceding fall and stored in the laboratory during the winter

The red-clover seeds which were gathered in the spring consisted of eight lots from heads which remained intact upon the parent plants and were several inches above the ground, and eight other lots from heads which were embedded in the mud.

The alsike-clover, sweet-clover, and yellow-trefoil seeds gathered in the spring were all embedded in the mud. A large number both of these and of the red-clover seeds which were embedded in the mud had germinated and produced a dense growth of strong green seedlings before they were gathered. Many red-clover seeds had softened, and a few had germinated even in the heads which were several inches above the soil. About half an inch of soil was taken up with the seedlings which were growing in the soil, and carefully worked over to re-

move all the seeds. All seeds which had softened and looked healthy were counted as germinated.

The red-clover seeds which were impermeable when removed from the soil or from the seed heads were subjected to a germination test for one month in a chamber at about 20° C. The seeds which germinated in the germinating chamber were included with those which germinated in the soil in determining the germinating capacity.

All the alfalfa seeds gathered in the spring were from pods which remained upon the straw a foot or more above the soil. Ninety per cent or more of the seeds in these pods were brown and dead, and some were partly disintegrated. The remaining 10 per cent or less were bright, plump, and yellow. These were retained for the germination test. All alfalfa seeds which could be found in the surface soil under the plants were dead.

There were included in the examination 9,723 red-clover seeds, 575 alsike-clover seeds, 412 sweet-clover seeds, 200 alfalfa seeds, and 99 yellow-trefoil seeds, which were gathered in the spring.

Table X gives the results of the investigations.

TABLE X.—*Germination of leguminous seeds after passing the winter on or under the parent plants in the field compared with the germination of seeds harvested the previous fall*

| Kind of seed. | Number of lots. | Season in which gathered. | Average percentage of— | | | Calculated average percentage of the seeds previously impermeable which became permeable. |
|---|---|---|---|---|---|---|
| | | | Germination. | Impermeable seeds. | Dead seeds. | |
| Red clover | 8 | Fall, 1909 | 8 | 88 | 4 | 1 |
| Do | 8 | March, 1910[a] | 66 | 33 | 1 | 63 |
| Do | 8 | March, 1910[b] | 37 | 61 | 2 | 30 |
| Alsike clover | 1 | Fall, 1912 | 15 | 84 | 1 | −8 |
| Do | 1 | April, 1913[c] | 59 | 37 | 4 | 52 |
| Sweet clover | 1 | Fall, 1912 | 10 | 90 | 0 | −2 |
| Do | 1 | April, 1913[c] | 63 | 36 | 1 | 59 |
| Alfalfa | 1 | Fall, 1913 | 76 | 22 | 2 | ..... |
| Do | 1 | April, 1914[d] | 3 | 97 | 0 | ..... |
| Yellow trefoil | 1 | April, 1913[c] | 64 | 36 | 0 | ..... |

[a] From heads embedded in the soil; germination reported includes 1 month in chamber.
[b] From heads on the straw above the soil; germination reported includes 1 month in chamber.
[c] Germination in the field only.
[d] From dry heads well above the soil; germination in chamber.

1. From 52 to 63 per cent of the clover and yellow-trefoil seeds which were impermeable in the fall softened after passing the winter in the soil. Of the impermeable red-clover seeds which were in heads several inches above the soil 30 per cent softened. In the meantime only 1 per cent of the impermeable red-clover seeds which were stored in the laboratory over the winter became permeable, and the percentage of alsike-clover

and sweet-clover seeds which were impermeable increased under dry storage in the laboratory. Nearly all of the seeds which softened after wintering in the soil or on the plants germinated.[1]

2. Only those alfalfa seeds which remained impermeable survived the winter on the plants. Only 3 per cent of those seeds gathered in the spring germinated and 97 per cent were impermeable. Of those gathered in the fall 76 per cent germinated and 22 per cent were impermeable, only 2 per cent being dead.

Additional tests were made upon self-sowed seed in February and March, 1916, taking advantage of the effect of a favorable alternation of temperatures upon the softening of the seeds (see p. 779–781), as follows:

One lot of self-sowed red-clover seed and two lots of self-sowed sweet-clover seed, with the soil in which they were embedded, were gathered on February 29 and immediately placed in an ice box in which the temperature was constantly somewhat below 10° C. During the next few days the seeds were separated from the soil without allowing them to become dry at any time. Both before and after removing them from the soil they were daily alternated between the ice box and the germinating chamber at 30°.

Many seeds had produced seedlings, and others had softened but had not germinated in the field. Many of the seed which were impermeable when taken into the laboratory softened in the next four days. After the fourth day there was very little change during the following three weeks, although the seeds were incubated in the ice box for nine days and then again alternated between the ice box and the chamber at 30° C.

The numbers of seeds and seedlings recovered from the soil were as follows: Of red clover, 4,610; of the two lots of sweet clover, 1,508 and and 980, respectively. By the end of the fourth day after collecting the seeds, 86, 54, and 66 per cent of these different lots had softened either in the field or in the laboratory. If it be assumed that 90 per cent of these seeds were impermeable the preceding fall, it can be calculated that 84, 49, and 62 per cent of the impermeable seeds softened.

Besides the leguminous seeds already considered, eight lots of okra seeds were gathered in April, 1913, after passing the winter in the field. The great majority of these seeds were dead, but the percentage of dead seeds varied according to the previous exposure, being 69 per cent of the seeds in closed pods on the ground, 91 per cent of the seeds in closed pods on the stalks, 95 per cent of the seeds in opened pods on the stalks, 99 per cent of the seeds in opened pods on the ground, and all of the shelled seeds lying loose on the ground. Of the seeds which softened without clipping none germinated except of those which had been wintered in closed pods on the ground, where they had the full protection

[1] In this connection Hume's observations on sweet clover in South Dakota are interesting (12). Unhulled sweet-clover seed was sowed in August, 1911 and in 1912. Only a few seedlings were produced the year the seeds were sowed, but in each case a good stand of sweet-clover plants came up the following spring.

of the pod and a part of the time the protection of a snow cover. Of seeds which were gathered from the same cultivated rows the preceding fall and stored in the laboratory, 23 per cent germinated, 71 per cent were impermeable, and only 6 per cent were dead.

### PRODUCTION OF SEEDLINGS BY IMPERMEABLE SEEDS DURING ONE YEAR IN GREENHOUSE FLATS WITH FREEZING AND THAWING

Seeds of a number of lots of red clover, alsike clover, white clover, sweet clover, crimson clover, alfalfa, and okra were sowed in rows in greenhouse flats on March 18, 1911, and the tests were continued for 12 months.

From November 18 to December 11 and again from December 20 to January 25 the flats were outdoors. Each of these periods included some very cold days during which the soil in the flats became thoroughly frozen.

During the eight months previous to November 18, during the nine days between the two outdoor periods, and again from the end of the second period out of doors on January 25 to the end of the experiment on March 19, 1912, the flats were kept in a greenhouse.

At the end of the first 11 days with the clovers and alfalfa and at the end of the first 22 days with okra the percentages of seedling production in the greenhouse flats were approximately the same as the percentages of germination in a germinating chamber in, respectively, 4 and 10 days. All seedlings which appeared after the first 11 or 22 days were considered as being produced by impermeable seeds.

Although the experiment was continued in the greenhouse for nearly two months after the end of the second period of freezing, very few seedlings were produced after the first week of that time.

At the end of the experiment the soil was dried, broken up, and sifted through sieves of the proper sizes, and as many as possible of the seeds which still remained impermeable were recovered.

TABLE XI.—*Production of seedlings by impermeable clover, alfalfa, and okra seeds when submitted to freezing and thawing*

| Kind of seed. | Number of impermeable seeds found in chamber test. | Calculated percentages of the impermeable seeds which produced seedlings. | | Percentages of the impermeable seeds— | |
|---|---|---|---|---|---|
| | | In eight months before freezing. | After freezing. | Recovered from the soil. | Decayed or lost. |
| Red clover | 100 | 20 | 28 | 7 | 45 |
| Alsike clover | 52 | 4 | 23 | 9 | 64 |
| White clover | 136 | 3 | 15 | 21 | 61 |
| Sweet clover | 448 | 5 | 61 | 11 | 23 |
| Alfalfa | 162 | 37 | 4 | 2 | 57 |
| Crimson clover | 44 | 50 | 0 | 0 | 50 |
| Okra | 340 | 38 | 1 | 12 | 49 |

Table XI shows the calculated percentages of the impermeable seeds which produced seedlings before and after the freezing of the soil, the percentages which were recovered from the soil after the experiment, and the percentages which decayed or were lost.

1. From one-third to one-half of the impermeable seeds of alfalfa, crimson clover, and okra produced seedlings during the first eight months, while the flats remained in the greenhouse, but no crimson-clover seedlings and only a few seedlings of the other species appeared after the periods of freezing.

2. A small percentage of the impermeable seeds of red clover, alsike clover, white clover, and sweet clover produced seedlings in the first eight months, and a considerably larger percentage after the freezing of the soil. The seedling production after the freezing of the soil was particularly large (61 per cent) with sweet clover.

3. Only small percentages of the seeds were recovered from the soil at the close of the experiment. Approximately one-fourth of the sweet-clover seeds and approximately one-half of the seeds of the other species of plants were unaccounted for. While undoubtedly a few of these were lost, the majority of them must have softened and decayed during the experiment. The surface of the soil was at times crusted, and toward the end of the experiment much of it was thickly overgrown with moss. These conditions probably prevented some seedlings from reaching the surface even when the seeds germinated normally.

## PRODUCTION OF LEGUMINOUS SEEDLINGS IN THE FIELD COMPARED WITH GERMINATION IN A GERMINATING CHAMBER

In May, 1912, and again in May, 1913, field tests were conducted in comparison with chamber germination tests. The soil used was a rich sandy loam which held water well and was easily pierced by the young seedlings.

Table XII shows the percentages of impermeable seeds (determined in chamber test), the percentages of chamber germination in from four to eight days, and the percentages of seedling production in from ten to twenty days.

1. In the tests of 1912 the percentages of germination were greater than the percentages of seedling production in the field. However, those lots which contained small percentages of impermeable seeds produced much larger percentages of seedlings than those lots which contained large percentages of impermeable seeds.

2. There was a striking difference between those stands of plants secured from lots of impermeable seed and those stands secured from lots of seed but few of which were impermeable. Plate CVI shows this difference for alsike-clover and white-clover plants produced in 1912. The photographs were taken about four months after the seeds were

planted. In each photograph the two side rows were from one lot of seed over 90 per cent of which germinated in the chamber (Table XII, No. 146785 and 145571), and the middle row, with only a few scattered plants, was from a lot of seed over 90 per cent of which was impermeable (Table XII, No. 140624 and 140670). The same number of seeds was planted in each row and both lots of each kind of seed were planted on the same day.

TABLE XII.—*Seedling production by leguminous seeds in the field compared with germination in a germinating chamber*

| Kind of seed and year in which test was made. | Test No. | Percentage of impermeable seeds according to chamber test. | Percentage of germination in chamber in from four to eight days. | Percentage of seedlings in field in from ten to twenty days. |
|---|---|---|---|---|
| 1912. | | | | |
| Red clover | 83843 | 54 | 46 | 38 |
| | 85371 | 65 | 34 | 29 |
| | 85454–0 | 38 | 62 | 49 |
| | 85454–1 | 72 | 28 | 28 |
| | 124135 | 81 | 18 | 8 |
| | 146458 | 2 | 94 | 67 |
| | 146582 | 1 | 95 | 59 |
| Alsike clover | 140624 | 94 | 4 | 3 |
| | 146785 | 3 | 96 | 80 |
| White clover | 140670 | 96 | 4 | 3 |
| | 145571 | 5 | 94 | 69 |
| Sweet clover | 29406 | 97 | 3 | 5 |
| | 123687 | 3 | 45 | 28 |
| Alfalfa | 145394 | 20 | 80 | 52 |
| | 146673 | 0 | 97 | 65 |
| 1913. | | | | |
| Red clover | 82955 | 78 | 22 | 32 |
| | 149080 | 4 | 96 | 93 |
| Alsike clover | 140624 | 94 | 3 | 3 |
| | 148714 | 9 | 90 | 80 |
| White clover | 140670 | 95 | 3 | 7 |
| | 148502 | 4 | 94 | 83 |
| Sweet clover | 78539 | 87 | 6 | 9 |
| | 119982 | 16 | 67 | 64 |
| Alfalfa | G 1 .... | 71 | 25 | 44 |
| | 149305 | 3 | 96 | 84 |
| Hairy vetch | A 1 .... | 5 | 85 | 73 |

3. Figure 5 represents graphically for each lot of seed used in 1913 the percentage of germination in 8 days, the percentage of seedlings produced in the field in from 16 to 18 days, and the sum of percentage of germination and percentage of impermeable seeds. The space between the line at the top of the figure (germination plus impermeable seeds) and the lowest line in the figure (chamber germination) represents the percentage of impermeable seeds in each lot. The line representing chamber germination crosses the line representing seedling production at a point which corresponds to 60 per cent of germination, with nearly all

of the other 40 per cent of the seeds impermeable. When more than 40 per cent of the seeds in a lot were impermeable the percentage of seedling production was greater than the percentage of chamber germination; when less than 40 per cent were impermeable, chamber germination exceeded seedling production. We see here undoubtedly the combined

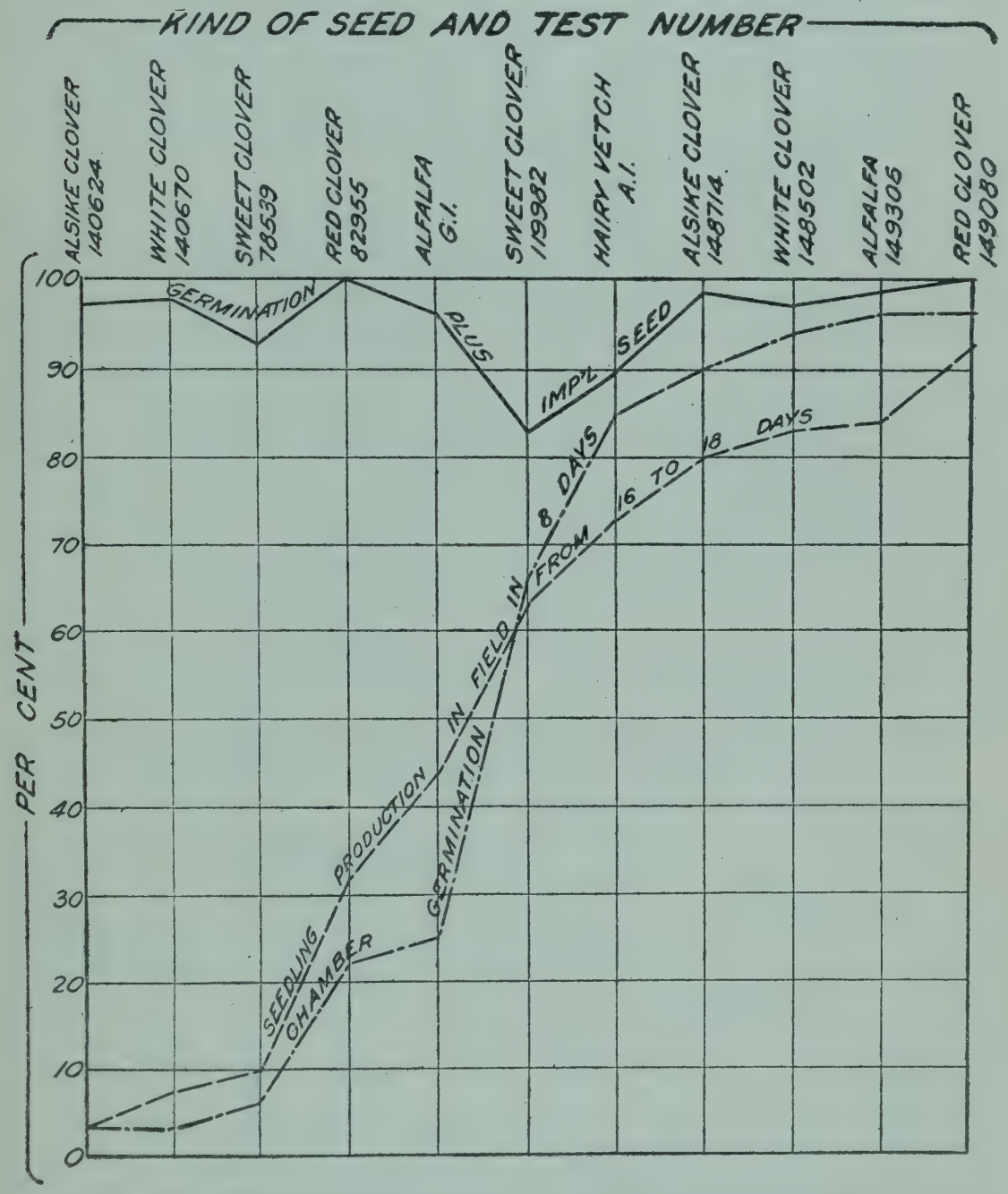


FIG. 5.—Curves of the seedling production in the field in 16 to 18 days and of the germination in chamber in 8 days.

effect of two separate conditions: First, a larger percentage of the impermeable seeds germinated in the field than in the chamber; second, some of the seeds which germinated in the soil did not produce seedlings which penetrated the overlying soil and were counted.

FIELD TESTS AND CHAMBER GERMINATION TESTS CONTINUED FOR ONE YEAR

The field tests begun in May, 1913, were kept under observation for nearly 13 months. In making these tests 500 seeds of each lot were spaced 2 inches apart in rows 4 inches apart in well-prepared beds which had been steam sterilized to kill weed seeds.

Observations were made frequently throughout the summer and as late as the middle of October. The plants were occasionally thinned to prevent crowding and to facilitate the observations. Early in November the beds were covered with cheesecloth on wooden frames to protect them from contamination by other seeds.

No observations were made after the cheesecloth covers were put in place until January 2 and 3 at the close of a period of warm, rainy weather. At this time there were a large number of new seedlings in the beds which were planted with lots of seed containing large percentages of impermeable seeds and a few in beds which were planted with lots of seed containing small percentages of impermeable seeds. Many of these seedlings had been heaved out by preceding freezes and there were evidences that some seedlings had been destroyed by insects. All of the seedlings which appeared in January and were not otherwise destroyed were killed by subsequent freezing and thawing.

By the 23d of March healthy clover seedlings of all kinds had appeared in abundance in protected places in the vicinity of the sterilized beds. On this day the cheesecloth covers were permanently removed.

TABLE XIII.—*Seedling production by impermeable clover and alfalfa seeds in the field in 12½ months compared with germination in a germinating chamber*

| Kind of seed. | Calculated percentages of impermeable seed which produced seedlings in field. | | | | | Percentages of impermeable seeds which germinated in chamber at room temperature in 12½ months. |
|---|---|---|---|---|---|---|
| | In first 16 to 18 days. | During season planted. | Following winter.[a] | Following spring.[b] | Total in 12½ months. | |
| Red clover | 13 | 17 | 18 | 39 | 74 | 31 |
| Alsike clover | 0 | 3 | 10 | 45 | 58 | 5 |
| White clover | 4 | 7 | [c]4 | [c]39 | 50 | 16 |
| Sweet clover | 4 | 8 | 17 | 27 | 52 | 7 |
| Alfalfa | 27 | 74 | 1 | ..... | 75 | 66 |

[a] These seedlings appearing in midwinter were killed by later freezing.
[b] Produced 1914 stand of plants.
[c] About three-fifths of the white-clover bed became so covered with the growth of plants produced in 1913 that observations the following winter and spring had to be confined to the other two-fifths of the bed. From these observations the percentages for the whole bed were calculated.

No new seedlings appeared during February, which was very cold, New growth began late in March and new seedlings appeared in increasing numbers from this time to about the middle of April and more slowly

thereafter until about the middle of May. Very few appeared later than May 20. Wherever these seedlings were densely shaded by healthy plants of the preceding season they grew tall and slender at first and ultimately disappeared.

Table XIII shows the calculated percentages of the impermeable seeds of lots originally containing large percentages of impermeable seeds which produced seedlings during the first 16 to 18 days, the first spring and summer, during the following winter, during the following spring, and the total percentages of impermeable seeds which produced seedlings in the field and which germinated in the chamber in 12½ months.

1. From 5 to 16 per cent of the impermeable seeds of alsike clover, white clover, and sweet clover and 31 per cent of the impermeable red-clover seeds germinated in the chamber in 12½ months. During the same time 74 per cent of the impermeable red-clover seeds and from 50 to 58 per cent of the impermeable seeds of the other kinds of clover produced seedlings in the field. Of these seedlings from one-twentieth to one-fourth appeared during the season in which the seeds were planted, from one-twelfth to one-third appeared during the following winter, but were killed before the winter was over, and the remainder, representing from one-fourth to one-half of the whole number of impermeable seeds planted, appeared in the spring of 1914 and produced the 1914 stand of plants.

2. Of the impermeable alfalfa seeds 74 per cent produced seedlings in the field during the first season and 1 per cent the following winter, while only 66 per cent germinated in the chamber in 12½ months. No observations were made on this lot in the field in the spring of 1914.

3. From one-fourth to one-half of the impermeable seeds of the different kinds either remained impermeable in the soil or softened and died.

Figure 6 shows graphically the percentages of seedling production in the field and of germination in chamber in 12½ months, and the sum of percentages of germination and of impermeable seeds according to chamber test. The different lots of seed are represented in the same order here as in figure 5, and the curve at the top of the figure is the same as occurs at the top of figure 5.

At the end of a year the seedling production by all the lots which contained large percentages of impermeable seeds had surpassed the germination in the chamber by an amount roughly proportional to the percentages of impermeable seeds which they contained. With all the lots which contained small percentages of impermeable seeds except sweet clover 119982 the percentages of seedling production in the field, even after 12½ months, were less than the percentages of chamber germination in eight days.

## SUMMARY OF SEEDLING PRODUCTION IN SOIL BY IMPERMEABLE CLOVER AND ALFALFA SEEDS

The results of a number of separate experiments on seedling production by impermeable seeds have been discussed in the preceding pages. The different series of tests were made at different times and with different lots of seed. Although this fact makes a direct comparison of the

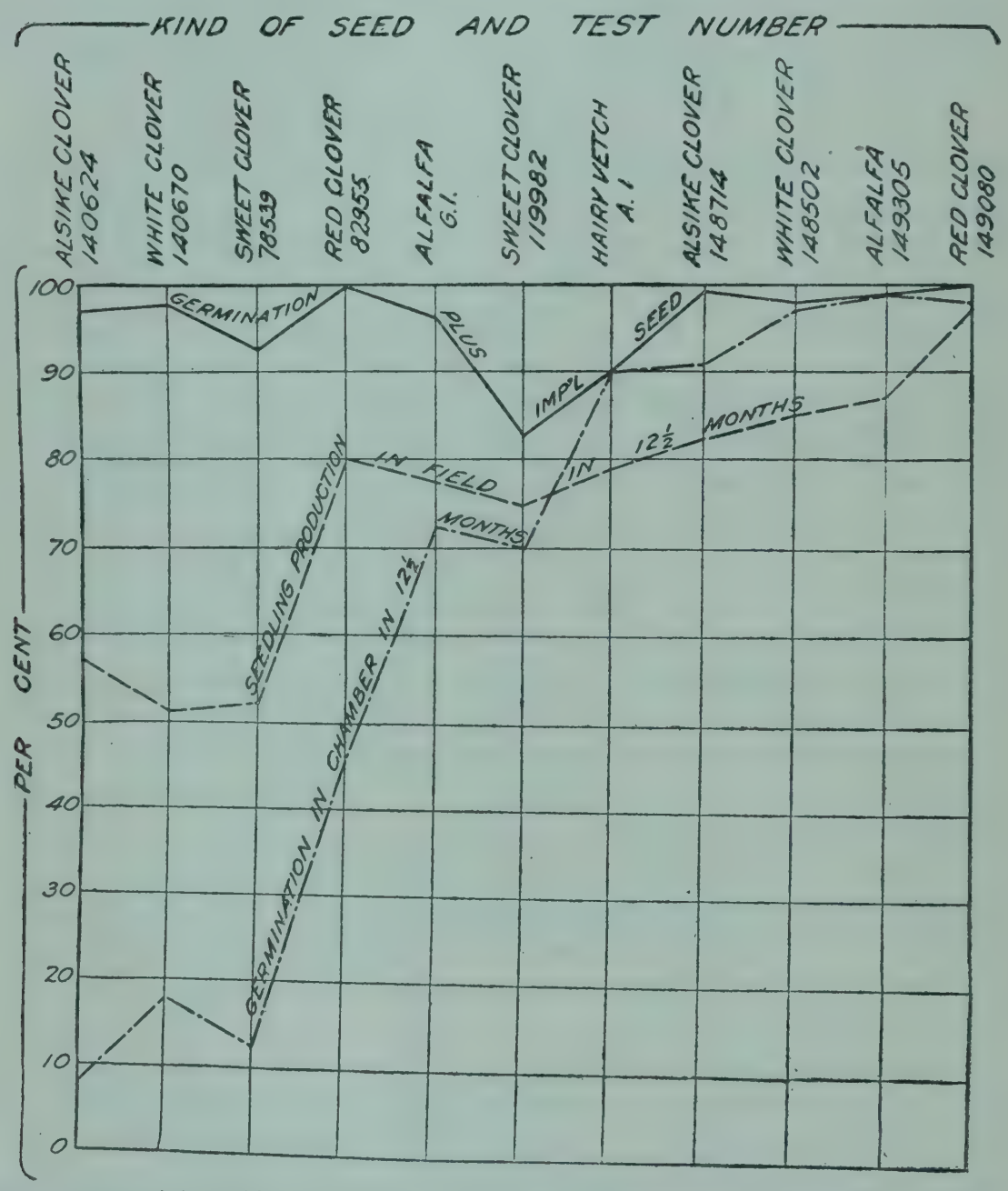


FIG. 6.—Curves of the seedling production in the field and of the germination in chamber in 12½ months.

results of the different series of tests impossible, some important considerations are emphasized by grouping the different series together, as in Table XIV. The results of check tests conducted in germinating chambers are included if such check tests were made; also the percentages of chamber germination of impermeable seeds in one year taken from Table III.

TABLE XIV.—*Production of seedlings by impermeable seeds; assembled results of different experiments*

| Place and description of test. | Duration of test. | Average percentage of the impermeable seeds which germinated or produced seedlings. | | | | |
|---|---|---|---|---|---|---|
| | | Red clover. | Alsike clover. | White clover. | Sweet clover. | Alfalfa.[a] |
| Greenhouse (p. 774) | 3 months... | 16 | ..... | 5 | 5 | 38 |
| Check in germination chamber, room temperature | ....do..... | 5 | ..... | 3 | 2 | 8 |
| Cold frame (Table VI) | 6 months... | 14 | ..... | ..... | 14 | [b] 80 |
| Cold frame, with freezing (Table VIII) | 4 months... | 38 | 50 | 30 | 74 | 56 |
| Check in germination chamber, room temperature | 3 months... | 5 | 12 | 3 | 2 | 4 |
| Seedlings produced in spring from seeds wintered in field (Table X). | ..... | [c] 63 | [d] 52 | ..... | [d] 59 | ..... |
| Seedlings produced in spring from seeds wintered in field (p. 23). | ..... | [c] 84 | ..... | ..... | [d] 49<br>[d] 62 | ..... |
| Cold frame and greenhouse with winter freezing (Table XI). | 1 year..... | [d] 48 | [d] 27 | [d] 18 | [d] 66 | [d] 41 |
| Field, in sterilized beds (Table XIII). | ....do ..... | [d] 74 | [d] 58 | [d] 50 | [d] 52 | [d] 75 |
| Check in germination chamber, room temperature. | ....do ..... | 31 | 5 | 16 | 7 | 66 |
| Germination chamber; average of several commercial lots (Table III) | ....do ..... | 27 | 21 | 13 | 21 | 70 |

[a] When alfalfa seeds were in a frozen medium during any part of the experiment, nearly all seedlings were produced before freezing occurred.
[b] In 2 months.
[c] Calculated from heads embedded in the soil.
[d] Calculated.

1. In each series of tests a large proportion of the impermeable alfalfa seeds produced seedlings in the soil. It should be remarked that in each series of tests nearly all of the seedlings produced from impermeable alfalfa seeds appeared during the first month or two of the experiment and that very few alfalfa seedlings appeared after the freezing of the soil except when the seeds were too cold to germinate from the beginning of the test period until after the freezing had occurred. (See Table VIII and accompanying text.) An examination of alfalfa seeds which had passed one winter under the parent plants shows that continued severe freezing and thawing in wet soil will soften and kill practically all (p. 783).

2. With each of the various kinds of clover the percentage of seedling production was small when no freezing of the soil occurred during the experiment.[1] Seedling production from impermeable clover seeds was greatly increased by the freezing of the soil and was greatest (except with sweet clover) in the series of field tests which were continued for a year.

In every series of experiments in which check tests were made in the germination chamber the average percentage of seedling production both of alfalfa and of the clovers was greater than the average percentage of

[1] In a few cases with individual lots of seed of alsike clover and white clover, a large percentage of the impermeable seeds produced seedlings in a short time in soil in a moderately warm greenhouse. These cases were so rare as to be almost negligible.

chamber germination. The differences were insignificant in some cases, but were very large in the field tests of the clovers continued for one year.

In this connection the effect of temperatures a few degrees above freezing should be emphasized. The laboratory tests in which certain alternations of temperature were used following cool constant temperatures show conclusively that actual freezing is not necessary in order to cause the subsequent softening and germination of many impermeable clover seeds (p. 781). Moreover, in many cases larger percentages of the impermeable seeds softened and germinated in these tests than in any of the tests with seeds which had passed the cold winter months in the soil under the parent plants (p. 781–784). These facts indicate that impermeable clover seeds would germinate as well if sowed several weeks before the beginning of warm weather in the spring as if sowed the preceding fall. In addition, spring sowing would avoid the danger of winter-killing softened seeds or young seedlings.

## USE OF IMPERMEABLE SEEDS

The value to the farmer of the impermeable seeds occurring in any lot of seed will vary according to the kind of seed, the germinating capacity, the percentage of impermeable seeds in the lot of seed under consideration, the age of the seed, and the time of sowing the seed.

Impermeable alfalfa seed sowed late in the spring is of more value to the crop than impermeable sweet-clover seed sowed at the same time.

If the percentage of impermeable seed in a given lot is small (10 per cent or less) and the rest of the lot consists of strong, germinable seeds, the impermeable seeds are of little importance both because of their fewness in comparison with the seeds which germinate readily and because of the varying quantities of seed which are sowed according to common practice. It is when the impermeable seeds constitute a large percentage of the seed in a given lot that their real value becomes a question of agricultural importance.

In seed that is several years old the viability of the permeable seeds may have become so low that the impermeable seeds, which lose their vitality more slowly, are relatively much more important than in lots of fresh, new seeds.

Impermeable clover seed sowed early in the spring is of more value than the same seed sowed later, when the weather has become settled and warm.

The following general rules, based upon the experimental results and upon the considerations just outlined, are suggested as guides in agricultural practice with the plants investigated.

Assuming that all seeds have been tested for germinating capacity and percentage of impermeable seeds, calculate the amount of seed to sow as specified below.

1. RED CLOVER, ALSIKE CLOVER, WHITE CLOVER, AND WHITE SWEET CLOVER

A. When seed is to be sowed in the late spring or summer.—Consider one-tenth of the impermeable seed as good. Add one-tenth of the percentage of impermeable seed to the percentage of germination. Calculate from this sum the quantity of seed of the given lot necessary to give the desired quantity of good germinable seed. For example: It is desired to sow per acre the equivalent of 15 pounds of viable seed none of which is impermeable. Fifty per cent of the lot of seed to be used germinates and 40 per cent is impermeable. To 50 per cent add one-tenth of 40 per cent, or 4 per cent. Consider 54 per cent as good. Then divide 15 by 0.54. The quotient, or 27.8, is the number of pounds of seed to sow per acre. In the form of an equation we have the following statement:

$$\frac{\text{Number of pounds good seed desired per acre}}{\text{Percentage of germination} + 1/10 \text{ the percentage of impermeable seed}} = \left\{\begin{array}{l}\text{Number of}\\ \text{pounds}\\ \text{to sow}\\ \text{per acre,}\end{array}\right.$$

$$\text{or } \frac{15}{0.50 + \frac{0.40}{10}} = 27.8$$

The impermeable seeds remaining in the ground will constitute a reserve which, under favorable conditions in a cold climate, will improve any thin areas in the stand the following spring. This, however, should not be counted upon, as spots not occupied by desirable plants before the second growing season will almost certainly be appropriated by more rapidly growing weeds unless the field is unusually free from weed seeds.

B. When seeding in the late fall or winter or in the spring a month or so before the end of freezing weather.—Consider all of the impermeable seeds as good. Add the percentage of impermeable seeds to the percentage of germination. Calculate from the sum the quantity of seed to be used, as under A. For instance, in the example given under A add 40 per cent to 50 per cent, which gives a total of 90 per cent. Then divide 15 by 0.9. The quotient, 16.7, is the number of pounds to sow per acre. Probably not all the impermeable seeds will soften and produce seedlings, but the seedlings produced by them will be less liable to injury than the seedlings produced by permeable seeds which soften immediately, germinate on the first warm days, and may be killed by subsequent freezing.

C. When seeding in the spring after danger of severe frost but a month or more before the end of cool weather.—Consider two-thirds of the impermeable seeds as good and proceed as under A and B.

2. ALFALFA AND CRIMSON CLOVER

To the percentage of germination add two-thirds of the percentage of impermeable seeds and calculate the quantity of seed to be used as given under red clover. More than two-thirds of the impermeable seeds may germinate, but not soon enough to compete with weeds.

3. HAIRY VETCH

To the percentage of germination add one-half of the percentage of impermeable seeds as a basis for calculating the quantity of seed to be used. Proceed as under red clover.

4. OKRA

To the percentage of germination add one-fourth of the percentage of impermeable seeds as a basis for determining the quantity of seed to sow, and proceed as under red clover. More than one-fourth of the impermeable seed will probably germinate, but too late to contribute to a uniform stand.

## CONCLUSION

By "impermeable seeds" is meant those seeds all parts of whose seed coats are impermeable to water at temperatures favorable for germination.

It is impossible to distinguish between impermeable and permeable seeds except by testing their ability to absorb water at a temperature favorable for germination.

The production of impermeable seeds is particularly characteristic of the Leguminosae, but it occurs also in many other plant families.

Among the cultivated species which sometimes produce impermeable seeds are okra, hollyhock, alfilaria, atriplex, asparagus, morning-glory, canna, cherry tomato, and nearly all of the cultivated species of Leguminosae.

Impermeable seeds frequently retain their vitality for many years, sometimes for at least as many as 80 years.

Fresh impermeable seeds germinate promptly when the seed coat is broken or becomes permeable.

The viability of fresh impermeable seeds is frequently greater than the viability of fresh seeds of the same species which are permeable.

Seeds of the common clovers, alfalfa, and hairy vetch which are impermeable at the end of three to five years under laboratory conditions of storage retain their vitality apparently unimpaired up to that time. The viability of the permeable seeds in the same lots decreases slightly in the second and third year and more in subsequent years.

In dry storage nearly all impermeable alsike-clover, white-clover, and sweet-clover seeds remain impermeable until at least 2 or 3 years old. Impermeable red-clover seeds become permeable gradually in dry storage, but from one-third to two-thirds of them may still be impermeable after four years. The majority of impermeable alfalfa and hairy-vetch seeds become permeable before they are 2 years old. Okra seeds become less permeable as their age increases.

In wet blotters nearly all impermeable alfalfa, crimson-clover, hairy-vetch, and okra seeds soften and germinate in one year, though a very few may remain impermeable even after three or four years. Impermeable seeds of red clover, alsike clover, white clover, and sweet clover soften and germinate more slowly, but with no uniformity as to rate. All germinate within one year in some cases, while in other cases over 50 per cent are still impermeable after four years.

Impermeable clover seeds which were thoroughly matured before harvesting soften and germinate more slowly under conditions favorable for germination than do impermeable seeds of the same species which were less well matured; they also become permeable more slowly in dry storage.

Impermeable seeds become permeable more rapidly in wet blotters than in dry storage.

It is impossible to estimate even approximately in advance the proportion of the impermeable seeds in any given lot which will germinate in any given length of time under ordinary germination conditions.

A widely variable proportion of the impermeable seeds of alfalfa, crimson clover, and the larger seeded commercial species included in this investigation produce seedlings promptly in the soil under greenhouse conditions or in the open field in warm weather. Only in exceptional cases is this true of the impermeable seeds of the clovers, other than crimson clover.

The use of aqueous extracts from soil has no effect, and alternate wetting and drying of the seeds has but little effect on the germination of impermeable seeds.

Within ordinary limits neither the depth of planting nor the firmness of the soil affects the germination of impermeable clover and alfalfa seeds under greenhouse conditions. These factors may affect the stand secured by preventing some of the seedlings from reaching the surface.

Storing impermeable clover and alfalfa seeds at a temperature of 50° C. for one day or at 45° for six months has little or no effect upon their germinating capacity or permeability.

In wet blotters a temperature of 36° very slightly increases the softening of the impermeable seeds, but also kills some of the seeds.

Freezing, when wet, causes the subsequent germination of many impermeable seeds, but may kill some seeds which had previously softened.

Any constant temperature from 1° C. to 30° has little effect upon the softening of impermeable clover seeds.

Alternations of temperature have but little effect on the softening and germination of impermeable clover and alfalfa seeds if none of the temperatures used in the alternation is cooler than 20° C.

Alternations of temperature cause the softening and germination of many impermeable clover seeds when a temperature of 10° or cooler is used in alternation with a temperature of 20° or warmer. The effect of such an alternation of temperature is greatly increased by previously exposing the seeds to germination conditions at a cool temperature (10° C. or cooler), and is decreased by previously exposing the seeds to germination conditions at a warm temperature (30°).

Even under the most favorable conditions only a small proportion of impermeable red-clover, alsike-clover, white-clover, and white sweet-clover seeds produces seedlings promptly in the soil when sowed in warm weather.

Impermeable seeds of red clover, alsike clover, white clover, and white sweet clover will pass the winter in the soil in a freezing climate without injury. At least 50 or 60 per cent of them may be expected to germinate in the soil the following spring unless a part of them germinate during warm weather in the winter. If this occurs, the seedlings produced in the winter are liable to be killed by subsequent freezing.

A large proportion of impermeable alfalfa, crimson-clover, okra, and hairy-vetch seeds will germinate in the soil during the first few months after planting, some of them early enough to be of importance to the crop.

Nearly all alfalfa and okra seeds, even if they are impermeable in the fall, are killed when they pass the winter in the soil or on the plants out of doors in a freezing climate. A small proportion of the impermeable alfalfa seeds survive with their vitality uninjured. Some of the okra seeds remain impermeable during the winter, but the majority even of those which remain impermeable are killed by the winter's exposure.

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## PLATE CVI

Fig. 1.—A row of alsike clover from impermeable seeds between two rows from permeable seeds.

Fig. 2.—A row of white clover from impermeable seeds between two rows from permeable seeds.

1

2

# MENDELISM OF SHORT EARS IN SHEEP

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Among the various features under observation in the experimental breeding carried on at this Station with sheep the "short" ear trait is a very clear example of a simple Mendelian unit factor.

Short ears as referred to here are of a distinctive type with nearly straight lines running from the base and forming an abrupt, sharp point. They are also somewhat thicker than the ordinary type of ear. The longest of these ears so far observed in a mature animal measure 7 cm. (2¾ inches). Length as a character therefore forms quite a distinctive contrast [1] between this type and that of Rambouillet ears, which measure about 11.5 cm. (4½ inches); Southdowns, which measure about 9.5 cm. (3¾ inches); and Shropshires and native, which measure about 10 cm. (4 inches). In fact, all ordinary ear lengths observed among various breeds and types seem to run close around 10 cm. (4 inches) or over.

The experimental data given here are derived from one native ewe and her progeny, which number 15 head. This ewe was purchased from a neighboring farm with 19 other native ewes, none of which had short ears or short-ear offspring. The character of her dam is unknown, but her sire is known to have possessed long ears. She was therefore in all probability simplex as to the character of ear length. This short-ear ewe (No. 69) was bred to a Hampshire ram (No. 3) for three successive years, producing three female offspring, all short-eared. Two of these died, leaving only one (No. 127). As no $F_1$ males of this type were available, she was used twice on a back cross with her sire (who was a pure long ear) and once on a similar back cross with No. 361 (also a pure long ear). From this cross three sets of twins were obtained with an equal number of short and long ears, which corresponds to the results expected from a back cross of a simplex to the recessive parent in a simple Mendelian unit character. She was later bred to her own son (No. 255), an offspring of this back cross, who showed the recessive trait. Being recessive, he should have been pure to long ears, and the cross on his dam should give similar results as the former matings of 127 with pure long-eared sires (No. 3 and 361). The actual result was one pair of twins, including one short-eared and one long-eared individual. This gave a total of four short-eared and four long-eared offspring from simplex × recessive parents.[2]

[1] No intermediate types either as to length, shape, or thickness have so far appeared.

[2] One mating was simplex × extracted recessive.

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The next type of mating was made with a short-eared male (No. 422), who was one of the pair of twins out of No. 127 by her son, and therefore her grandson. No. 422 was therefore simplex. He was bred to female 256, a simplex offspring of the first back cross (No. 3 × 127), and also to 127, who was simplex. These matings, being simplex × simplex, correspond to a mating of $F_1$ and should give the 3 to 1 ratio. Four offspring were obtained from these matings, three of which had short ears and one long, thus giving results again conforming numerically to theory as regards segregation of a simple Mendelian dominant character. The following diagram shows the various matings and their results. S indicates short ear; L, long ear.

$$F^1 \text{ offspring} \left\{ \frac{\text{Parent } ♂\ 3L \times \text{Parent } ♀\ 69S}{♀\ 127S \quad ♀\ 222S \quad ♀\ 254S} = 3S : 0L \right.$$

$$\text{Back cross: Simplex} \times \text{recessive} \left\{ \frac{♂\ 3L \times ♀\ 127S}{♂\ 255L \quad ♀\ 256S \quad ♀\ 313S \quad ♀\ 314S} \quad \frac{♂\ 361L \times ♀\ 127S}{♀\ 459L \quad ♀\ 460L} = 3S : 3L \right.$$

$$\text{Cross: Simplex} \times \text{extracted recessive} \left\{ \frac{♂\ 255L \times ♀\ 127S}{♂\ 422S \quad ♂\ 423L} = 1S : 1L \right.$$

$$\text{Cross: Simplex} \times \text{simplex} \left\{ \frac{♂\ 422S \times ♀\ 256S}{♀\ 573S \quad ♀\ 461S \quad ♀\ 462L} \quad \frac{♂\ 422S \times ♀\ 127S}{♀\ 572S} = 3S : 1L \right.$$

While the experiment has been discontinued at this Station, a few more data have become available this spring, as No. 127 was again bred to a pure long-eared ram, though with the primary purpose of studying her performance as a twin bearer. She again dropped twins, a long-eared and a short-eared individual, which further establishes her simplex character with regard to the short-ear trait. No. 462 dropped her first lamb this year. As she is a pure recessive and bred to long-eared sire, a long-eared offspring was the result, as expected.

No. 422 and 572 were sent to Dr. C. B. Davenport,[1] of the Station for Experimental Evolution, of the Carnegie Institute, who bred the two and also bred the male to 12 long-eared females. Dr. Davenport reports that the 12 ewes bred to No. 422 all lambed, 10 of them having dropped twins and 2 of them triplets, the short-ear trait appearing in about one-half of the offspring, which supports previous data indicating his simplex character with regard to short ears.

[1] Acknowledgments are due to Dr. Davenport for valuable advice given during the prosecution of this work.

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# LIFE-HISTORY STUDIES OF CIRPHIS UNIPUNCTA, THE TRUE ARMY WORM

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## INTRODUCTION

For an insect so commonly injurious and widely known as the army worm (*Heliophila*) *Cirphis unipuncta* Haworth, comparatively little concerning its life economy is recorded, and the detailed experiments herein reported represent phases of the more exact studies begun by this branch of the Bureau of Entomology.

Late in July, 1914, the army worm appeared in very destructive numbers in Huron and Sanilac Counties, Mich., and from the progeny of larvæ collected in that locality eggs and larvæ were obtained with which a series of molting and feeding experiments was started at La Fayette, Ind. The feeding experiments, where exact records of the amount of corn foliage eaten in each instar were kept, are especially interesting and instructive, for it will be noticed that more than 80 per cent of all the foliage eaten during the entire life of the larva was consumed during the last larval instar, which corroborates previous field observations to the effect that army worms rarely become evident and destructive until they are nearly full grown.

## GENERATIONS OF THE ARMY WORM

During the past year (1915) *Cirphis unipuncta* was bred continuously throughout the season from moths collected from May 13 to 15, to determine the average number of generations annually. Moths of *C. unipuncta* were first observed at La Fayette the night of May 13 feeding on the honeydew produced by *Pulvinaria vitis* L.,[1] *Lecanium quercifex* Fitch,[1] and *Callipterus discolor* Monl. on white oak, and it is quite likely that these moths were the adults of larvæ overwintering in this latitude. Moths were placed in large breeding cages under natural outdoor conditions, and eggs were laid and the larvæ first observed on June 7.

[1] Kindly determined for the Bureau of Entomology by Prof. J. G. Sanders.

Pupæ were found in the cage on June 27 and the adult moths began to issue on July 8. The generation series was continued in another cage in which moths issued from July 8 to 10, eggs were found on July 14, larvæ as early as July 20, and the first adults on August 30. These adults, issuing between August 30 and September 8, were similarly confined, and eggs were first noticed on September 25 and larvæ on September 28. During the winter of 1915–16 they survived as partially grown larvæ and completed their growth in April, 1916. Thus, it is observed that in the latitude of La Fayette three complete generations may occur annually, and from numerous observations of 1914 and 1915 it is evident that in some seasons a partial fourth generation may be present. Likewise, the overlapping of generations may account for a partial fourth, although a complete fourth generation is seldom, if ever, produced in this latitude.

## MOLTING AND FEEDING HABITS

The records of molts and of foliage eaten were made with larvæ confined in individual cages, three types of cages being used, namely, tin boxes, glass test tubes, and lantern globes (Pl. CVII, *A*). The tin boxes used for individuals 1 to 48 and 77 to 132 were of the common salve-box type, a 1-ounce size being used for the earlier stages and a 3-ounce size after the larvæ were about half grown. Individuals 49 to 76, inclusive, were reared in ordinary lantern-globe cages, with cheesecloth tops, placed on paper-padded saucers and containing corn foliage in bottles of water. Individuals 133 to 153 were reared in cotton-stoppered test tubes, which measured approximately 6 inches in length and 1 inch in diameter, smaller vials having been used for the first few instars. A single larva recently hatched was placed in each cage and fresh corn foliage given it as necessary. Frequent examinations were made to obtain as nearly as possible the exact hour of molting. Foliage uneaten was pressed, and from this the total amount eaten was computed for each larval instar, using for this purpose ordinary plotting paper squared to hundredths of an inch, by means of which a fairly accurate record of foliage eaten to thousandths of a square inch was obtained.[1]

The tin-box cages, 1 to 48 and 77 to 103, were kept indoors, and the lantern-globe cages, 49 to 76, and vial cages, 133 to 153, were kept on a latticed porch and were therefore under more nearly normal outdoor conditions. As the experiments were conducted for the most part during September and October, the nights were much cooler for the individuals on the porch, resulting in a noticeably longer life-cycle period for these than for the individuals kept indoors, where the coolness of the nights was much less evident.

[1] The authors take this occasion to acknowledge their indebtedness to Mr. D. G. Tower, of the Office of Cereal and Forage Insect Investigations, who assisted in measuring the pressed foliage and in making counts of eggs in the bodies of the moths.

For a summary of the data relative to the length of the various stages of *Cirphis unipuncta* and for the amount of foliage eaten in each instar, the reader is referred to Table I.[1] It will be noticed that the average lengths of the different stages for individuals in the lantern-globe cages and in glass vials were noticeably longer than for individuals reared in the tin boxes. This was not due to the difference in the style of cage, but can be accounted for, as mentioned above, by the fact that the lantern-globe and glass-vial cages were kept on a latticed porch, where the night temperature was much lower than in the laboratory where the tin boxes were kept. It will be noticed that the length of the egg stage is uniform in all cases, as they were kept under like conditions, and the average for 153 individuals was between approximately 5½ and 6¼ days. As will be noticed, the length of the first five larval instars did not vary greatly one

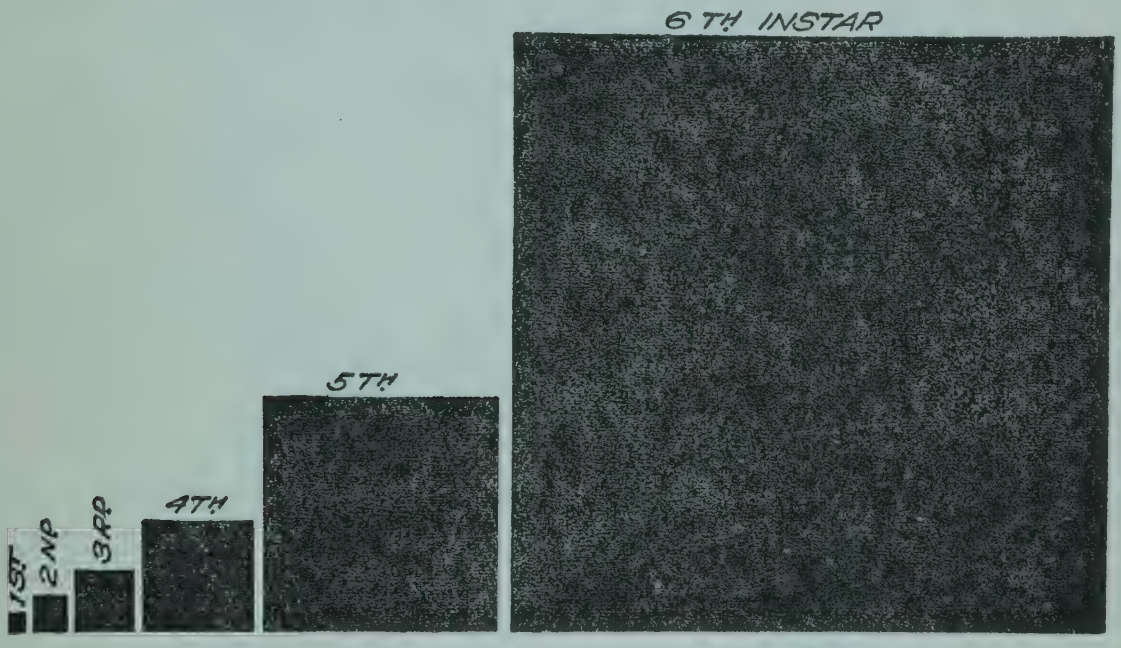



FIG. 1.—Diagram of relative amounts of foliage eaten in each larval instar by *Cirphis unipuncta*.

from the other, although the amount of foliage eaten in each of these instars was a gradual increase from nearly 0.03 of a square inch for the first instar to over 5 square inches for the fifth instar. The period for the sixth larval instar was noticeably longer than in any of the previous instars, being approximately two and one-half times as long; and the amount of foliage eaten in this instar was nearly seven times as much as in the fifth instar, and more than 80 per cent of all of the foliage eaten during the entire larval period. In other words, the total amount of foliage eaten by the larva during its entire life, an average from 108 individuals, was 41.394 square inches, and the average for the same individuals for the sixth instar alone was 34.128 square inches (see Table I and fig. 1). The remarkable voracity of the army worm during its last larval instar explains its sudden appearance in such enormous and destructive numbers only when it is nearly full grown.

[1] Owing to the size of the tables giving complete data for each individual, it is impossible to include them with this paper, but they are on file and may be obtained for reference by anyone interested.

TABLE I.—*Comparison of the molting and feeding of Cirphis unipuncta at La Fayette, Ind., from August to November, 1914*

| Cage No. and date eggs were laid. | Egg stage. | | First instar. | | | | Second instar. | | | | Third instar. | | | | Fourth instar. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Corn foliage eaten. | | | | Corn foliage eaten. | | | | Corn foliage eaten. | | | | Corn foliage eaten. | |
| | Number of individuals. | Duration. | Number of individuals. | Duration. | Number of individuals. | Average area. | Number of individuals. | Duration. | Number of individuals. | Average area. | Number of individuals. | Duration. | Number of individuals. | Average area. | Number of individuals. | Duration. | Number of individuals. | Average area. |
| | | *Hours.* | | *Hours.* | | *Sq. in.* | | *Hours.* | | *Sq. in.* | | *Hours.* | | *Sq. in.* | | *Hours.* | | *Sq. in.* |
| 1 to 48 (tin boxes): Aug. 22, 9.30 p. m | 48 | 136.5 to 157.5 | 40 | 78.707 to 98.891 | ..... | ..... | 36 | 53.219 to 65.254 | ..... | ..... | 36 | 56.236 to 72.125 | 35 | 0.338 | 32 | 78.383 to 89.883 | 31 | 1.305 |
| 49 to 76 (lantern globes): Aug. 26, 10 p. m., to Aug. 27, 10.30 a. m | 28 | 127.5 to 154.75 | 22 | 88.954 to 114.216 | 20 | 0.041 | 21 | 96.614 to 104.983 | 21 | .121 | 21 | 105.426 to 111.906 | 26 | .346 | 21 | 68.880 to 75.928 | 21 | 1.159 |
| 77 to 103 (tin boxes): Aug. 27, 9.45 p. m | 27 | 140.5 to 143.33 | 26 | 94.461 to 101.221 | 26 | .025 | 26 | 76.164 to 85.482 | 26 | .077 | 26 | 85.502 to 96.402 | 26 | .323 | 25 | 55.510 to 66.906 | 25 | 1.165 |
| 104 to 132 (tin boxes): Aug. 28, 6 p. m., to Aug. 29, 6 a. m | 29 | 135.25 to 158.50 | 25 | 83.980 to 101.920 | 25 | .025 | 25 | 93.400 to 103.980 | 25 | .096 | 24 | 72.229 to 81.573 | 24 | .351 | 24 | 51.208 to 66.052 | 24 | 1.214 |
| 133 to 153 (glass vials): Aug. 29, 9.30 p. m., to Aug. 30, 10.30 a. m | 21 | 128 to 131 | 19 | 126.284 to 142.250 | 19 | .031 | 16 | 94.500 to 100.578 | 16 | .134 | 15 | 51.733 to 62.666 | 15 | .339 | 14 | 58.393 to 70.285 | 14 | 1.101 |
| Mean | ..... | 134.11 to 151.05 | ..... | 91.366 to 107.012 | ..... | .0294 | ..... | 79.452 to 88.589 | ..... | .103 | ..... | 73.532 to 84.838 | ..... | .338 | ..... | 63.698 to 75.109 | ..... | 1.204 |
| Total | 153 | ..... | 132 | ..... | 90 | ..... | 124 | ..... | 88 | ..... | 122 | ..... | 121 | ..... | 116 | ..... | 115 | ..... |

TABLE I.—*Comparison of the molting and feeding of Cirphis unipuncta at La Fayette, Ind., from August to November, 1914*—Continued.

| Cage No. and date eggs were laid. | Fifth instar. | | | Sixth instar. | | | | Total corn foliage eaten. | | Total length of larval stage. | | Total length of pupal stage. | | Total length of life cycle. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of individuals. | Duration. | Average area of corn foliage eaten. | Number of individuals. | Duration. | Corn foliage eaten. | | Number of individuals. | Average area. | Number of individuals. | Duration. | Number of individuals. | Duration. | Number of individuals. | Duration. |
| | | | | | | Number of individuals. | Average area. | | | | | | | | |
| | | *Hours.* | *Sq. in.* | | *Hours.* | | *Sq. in.* | | *Sq. in.* | | *Hours.* | | *Hours.* | | *Hours.* |
| 1 to 48 (tin boxes): Aug. 22, 9.30 p. m. | 29 | 107.784 to 116.250 | 4.776 | 24 | 134.104 to 144.066 | 29 | 30.262 | [a] 29 | 36.681 | 24 | 537.6 to 561.6 | 24 | 387.780 to 393.780 | 29 | 1,084.320 to 1,089.993 |
| 49 to 76 (lantern globes): Aug. 26, 10 p. m., to Aug. 27, 10.30 a. m. | 21 | 96.583 to 106.428 | 5.354 | 19 | 301.287 to 311.274 | 20 | 28.26 | 19 | 35.281 | 20 | 786.864 to 803.4 | 19 | 702.864 to 716.304 | 19 | 1,632.144 to 1,656.312 |
| 77 to 103 (tin boxes): Aug. 27, 9.45 p. m. | 25 | 71.048 to 81.835 | 5.528 | 25 | 167.357 to 174.971 | 25 | 35.82 | 25 | 42.938 | 25 | 575.424 to 600.205 | 25 | 427.056 to 431.277 | 25 | 1,149.048 to 1,150.392 |
| 104 to 132 (tin boxes): Aug. 28, 6 p. m., to Aug. 29, 6 a. m. | 24 | 63.531 to 85.382 | 5.794 | 22 | 179.405 to 190.716 | 23 | 37.97 | 21 | 45.450 | 21 | 574.550 to 587.102 | 21 | 429.312 to 433.272 | 21 | 1,151.112 to 1,165.752 |
| 133 to 153 (glass vials): Aug. 29, 9.30 p. m., to Aug. 30, 10.30 a. m. | 13 | 76.725 to 80.515 | 5.58 | 13 | 297.675 to 301.618 | 13 | 40.982 | 13 | 48.167 | 13 | 726.264 to 742.902 | 13 | 761.928 to 768.768 | 13 | 1,632.840 to 1,616.848 |
| Mean | ..... | 81.756 to 91.822 | 5.364 | ..... | 203.336 to 212.261 | ..... | 34.128 | ..... | 41.394 | ..... | 626.52 to 641.333 | ..... | 512.328 to 518.976 | ..... | 1,276.488 to 1,287.432 |
| Total | 112 | ..... | ..... | 103 | ..... | 110 | ..... | 108 | ..... | 103 | ..... | 102 | ..... | 107 | ..... |

[a] This record does not include foliage eaten in first two instars.

It will be noticed that the egg stage approximated 6 days, the larval stage required about 26 days, and the pupal stage 21 days for over 100 individuals and that the average length of life cycle for these was 53 days. It will also be noticed that for the cages kept indoors, which would approximate late spring, summer, or early fall conditions, the total length of the larval period averaged about 23 days; that of the pupal period about 17 days, and that of the entire life cycle approximately 47 days; while for the individuals kept on the back porch, which approximated early spring or late fall conditions, the average length of the larval period was about 31 days, the pupal period 30 days, and the total life cycle approximately 68 days. The larva has six instars, molting five times previous to pupation. However, this may vary, for among the 107 individuals reared through to the adult stage one had seven instars, molting six times.

In the outbreak in Michigan, which claimed attention in the latter part of July, 1914, oats, barley, corn, grass, alfalfa, and beets were attacked in the order mentioned. Oats and barley were backward on account of an unusual June freeze and for this reason were more succulent and attractive to the army worms. In the case of small grains, especially oats, the relative amount of injury was much greater in proportion to the amount of actual food eaten than for such crops as corn, for in the former case the grain was clipped from the stalks by the worms, leaving most of the grain uneaten and the ground whitened with the grain heads.

At the time of the outbreak mentioned above the corn was 2 feet in height. Montgomery[1] has shown that mature corn plants have a foliage area of 927.8 to 1,912.9 square inches, with an average of 1,200 square inches. Corn plants 2 feet in height would have at the most not more than one-twelfth the foliage area of a mature plant; hence, it can be said with comparative assurance that a corn plant such as was found in Michigan during the 1914 outbreak would have not more than 100 square inches of foliage. Since one larva would eat 41.4 square inches, it would require five larvæ to devour two corn plants. With 8,890 corn plants to an acre (2½ plants to a hill and 3⅓ feet each way), it would require 21,473 worms to destroy an acre of corn 2 feet in height. Although seemingly a large number of worms, this number represents only the progeny of probably not more than 40 female moths.

According to the observations of the writers, the eggs laid at night in clusters of 25 to 134 on grass or other host plant between overlapping leaves fastened together or between the leaf sheaths, often none of the eggs or only a small part of the mass being visible (Pl. CVII, *B*). They are fixed to the leaf by means of a glutinous secretion which when dry is white and flaky. The largest number of eggs laid by a single female was 254 (see Table II), and in all cases where

[1] Montgomery, E. G. Correlation studies of corn. *In* Nebr. Agr. Exp. Sta. 24th Ann. Rpt., 1910, p. 108–159, illus. 1911.

the body of the dead female was examined many more eggs in all stages of development were found in the ovaries. In some cases more than 800 developed and undeveloped eggs were contained in the body of a single female.

The newly hatched larvæ first eat the eggshells, but apparently do not eat the white substance by which the eggs were attached. Later they feed on the tissues of the corn leaf on which they are resting, destroy the parenchyma, and leave the other leaf surface as a transparent membrane (Pl. CVII, *C*). Later, they feed from the edge of the leaf, devouring all the leaf tissue.

In the first instar the larvæ, if slightly disturbed, give themselves a compound twist, "humping" the body and drawing the head and thoracic segments around, bringing the ventral surface forward, and clinging by the pseudolegs and anal claspers. If further disturbed, they drop on a silken thread, and in this twisted position resemble balls of frass, losing all semblance of the larval form. Sometimes the larvæ contort themselves with a snap or fling, without the silken attachment, which fact seemed to explain the loss of an occasional individual in the experiments.

TABLE II.—*Eggs laid by individual females of Cirphis unipuncta at La Fayette, Ind., August to September, 1914*

| Cage No. | Aug. 26. | Aug. 27. | Aug. 28. | Aug. 29. | Aug. 30. | Aug. 31. | Sept. 1. | Sept. 2. | Sept. 3. | Sept. 4. | Sept. 5. | Sept. 9. | Total. | Eggs in body of dead female. | Total eggs laid and in body. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K[1] | ..... | ..... | ..... | 77 | 12 | (2) | ..... | ..... | ..... | ..... | ..... | ..... | 89 | (1) | ..... |
| L[1] | ..... | 28 | 5 | 90 | 20 | ..... | 4 | 71 | 36 | ..... | (2) | ..... | 254 | 91 | 345 |
| L[2] | ..... | ..... | ..... | 25 | 41 | 92 | 5 | 48 | 3 | (2) | ..... | ..... | 214 | 55 | 269 |
| L[3] | ..... | ..... | ..... | ..... | 134 | 39 | 48 | (2) | ..... | ..... | ..... | ..... | 221 | (1) | ..... |
| M[1] | 18 | 25 | 15<br>34<br>63 | 43 | 21 | (2) | ..... | ..... | ..... | ..... | ..... | ..... | 219 | 62 | 281 |
| M[2] | 15 | ..... | ..... | ..... | ..... | (2) | ..... | ..... | ..... | ..... | ..... | ..... | 15 | 431 | 446 |
| M[3] | ..... | ..... | ..... | 10 | ..... | ..... | 54 | ..... | ..... | ..... | ..... | (2) | 64 | (1) | ..... |

[1] Abdomen of dead moth not examined. [2] Died.

The use of a silk thread by larvæ when disturbed occurred with less frequency in the second instar. After dropping, the first-instar larvæ remain inactive for a moment before attempting to crawl away; and in later instars they swing the head or fore part of the body vigorously to one side and feign death. During the fifth molt, a disturbed larva feigned death for five minutes.

During the first and second instars the larvæ walk in a looping manner, but this characteristic is lost in the third and succeeding instars.

As the larva approaches a molt, the condition is recognizable by the largeness of the body diameter as contrasted with that of the head. When within a few hours of the molt, the larva habitually anchors its anal

claspers to whatever it is resting upon, usually a rigid object rather than the foliage. In some cases—for more than 14 hours in the third instar, 21 hours in the fourth instar, and 56 hours in the fifth instar—before the molt occurs, the apparent movement of the ocelli may be observed from their normal position to a position entirely behind the mask and within the stretched integument of the first thoracic segment. Before the rupture of the integument takes place, the old mask is in the position of a muzzle in relation to the withdrawn head. The mask separates from the body integument, which splits along the median dorsal line for perhaps four segments. The larva moves its head vigorously from side to side and brushes the mask off against some object or its own body. The withdrawal of the body from the integument begins with the muscular action of the body, the larva ultimately crawling forward a distance about one-fourth or one-half its length, and after resting thus for a time if undisturbed, it will almost always turn around and eat its newly molted skin. In no instance was a mask observed to be eaten. Immediately after molting, the head and anal segments are white, the body and cast skin moist, and the head noticeably larger in diameter than the body.

One larva was observed to eat its cast skin in four to four and one-fourth minutes. Another, which required only three minutes, held the cast skin with the front pair of legs, remaining stationary and pulling the skin to it, using the second pair of legs to hold to the foliage for the first minute and utilizing them to help manipulate the partly eaten skin. Another, in the third instar, took eight and one-half minutes to eat all but a trace of its freshly cast skin. It used its front legs almost continuously and the second pair of legs about half the time in holding the skin while eating it. Another had occupied about five minutes in this process when it was accidentally interrupted.

When the mature larva has finished feeding, the alimentary tract is soon emptied and shortens up to a marked degree, the larva then preparing to spin a thin cocoon. In nature the larva burrows into the ground or among or under trash. In the cages soil was supplied to some; others had only the paper on which they were lying, others were among corn foliage, and some had nothing whatever to utilize for a cell. In those instances where soil was supplied, the larva spun an appreciable quantity of silk as lining for its cell. Where paper was cut, the effort was appreciable, but not enough silk was used to form more than a shallow cup. Where there was foliage, it was chewed up and mixed with silk, but with scant resemblance to a cocoon; where no material was furnished, the silk was not evident, although pupation and the issuance of adults seemed to be equally normal.

Just before pupation a deep pit develops in the emargination of the posterior dorsal line of the mask, and transverse ridges on the dorsal portion of abdominal segments 5, 6, and 7 show distinctly through the larval skin. These ridges, interrupted at the ends, are marked with 20

to 22 blunt teeth, which appear as transverse striæ. Also the prothoracic spiracles of the pupa are observable just dorsad of those on the larva through the mesothoracic integument of the larva as red-brown chitinized spots. Two or three minutes before the skin begins to split, the dorsum of the second segment of the thorax had changed its shape conspicuously, suggesting a scutum or scutellum extension. The skin on the fifth and following abdominal segments appears to be shriveled up and ready to drop off about 15 minutes before pupation. A red mark observed on the clypeus of the new pupa is not observable through the larval mask.

Pupation, so far as the molt of the last larval skin is concerned, was observed to take place in about a minute. The integument splits along the median dorsal line of the thorax and the pupa vigorously works itself out. The mask splits along the inner seams of the genæ, leaving a triangular piece above the clypeus, and the whole remains clinging to the cast skin after the pupa has escaped. The lining of the esophagus was cast with the mask. The compound eyes continued as dark spots on the new pupa for several minutes after the mask had split. The wing pads at time of escape of pupa from exuvium—about four minutes after the mask first split—reached 8.25 mm. back from the apex of the head. The red-brown spot on the clypeus of the pupa is slightly above the point from which the esophagus was cast. There was no apparent function for this spot. The prothoracic spiracles of the pupa are red-brown, as evidenced through the larval skin a few minutes before pupation, and those posterior lack the red color but have slightly dusky rims. About 14 minutes after the molting the wing pads reached 9.60 mm. back from the apex of the head. From other observations the color of the prothoracic spiracles of the pupa may vary to crimson and other spiracles to pinkish red or rosy with a fine line of dark red at the rim. The pupa is at first cream-colored; in about 18 minutes after issuing it is a pale salmon, and in 25 minutes it begins to get brown. After that it browns up rapidly, but one and one-fourth hours afterwards it has not become full mahogany brown. The anal spines appear to be very useful in "kicking off" the exuvium. About 15 minutes after pupation the fat body shows through the wing pads and is irregularly assembled at ends of abdominal segments. About 30 minutes after pupation the two abdominal segments next behind the wing pads are getting rosy bands, and the last three segments are almost solidly the same color. The dorsum of the abdomen is of a dark-rose color, with two or three segments on the dorsal aspect having brown, roughened, chitinized edges. About 45 minutes after molting, the abdomen behind the wing pads is nearly uniformly rosy. The fat body now appears in rings through the wing pads. About one and one-fourth hours after pupation, the rose color gradually changes through dark salmon to light red-brown. The abdomen continues light red-brown for two and one-fourth hours after, but no red has begun to appear on the wing pads. Within three and

three-fourths hours the abdominal segments are nearly the normal shining red-brown.

Immediately after pupation the pupa stretches itself longitudinally almost to bursting, remains so from 12 to 15 minutes, and has been observed to contract and then extend itself again before resuming a natural appearance. On three pupæ immediately after pupation there was a transverse red-brown mark on the clypeus near its base and between the compound eyes. Each of these pupæ, while nearly white, showed a red-brown spot dorsad of the antennæ, which is the prothoracic spiracular spot.

The moth, immediately after emerging from the pupal shell, often carries a drop of clear fluid at its mouth. It then runs for a short distance. The wings require about 20 minutes to become fully expanded, with the upper surfaces folded together and hanging, and within one and a quarter hours they are in natural position flat on the back or slightly tectiform.

## DESCRIPTION OF STAGES[1]

### THE EGG

When first laid the eggs are perfectly smooth, shining milky white, and without any trace of sculpturing. Later the color changes through cream to flesh color, and just before hatching to a leaden cast. From a dorsal view they are apparently symmetrically spherical, but when laid in rows or masses, as is usually the case, they become compressed on two sides. Length, dorsal view, 0.542 to 0.561 mm.; width, dorsal view, 0.425 to 0.464 mm.

### THE LARVA

First instar.—Head pale vandyke brown, shining, its posterior margin deeply emarginate. Ocelli blackish, 12 in number, 6 on each side of the head, 5 of which are arranged in a semicircular form, the lowest of the 5, however, being separated by a space so that it appears paired with the sixth, which is located near the base of antenna. Cervical shield on prothoracic segment pale dusky. When first hatched the body is whitish, later becoming tinged with green, due to chlorophyll from the foliage eaten. Entire body sparsely clothed with moderately long fine hairs, which are about as long as half the width of the body, those projecting from the head and sides of the body whitish, the dorsal hairs blackish, and the body hairs on more or less conspicuous black tubercles. The fore pair of abdominal legs are somewhat atrophied, which may account for the characteristic looping walk of the first and second instar larvæ.

Just before the first molt the head is very dark brown to almost black, and the transverse cervical shield is dusky brown. There is a clear white, shining area with several blackish dots in a row on each side extending from the anterior end of the segment to a distance about equal to the width of the cervical shield, anterior to the latter, which represents the head and ocelli of the second-instar larvæ. The ground color of the body is of a decided greenish tint, paler toward the posterior end. The body segments bear seven alternate brown-ocher and white longitudinal stripes on each side of the whitish median dorsal line; the ventral surface is white. The dorsal lines are more or less obliterated or inconspicuous on the thoracic segments. Hairs much less conspicuous than early in the instar. The thoracic legs black, the pseudo and anal legs pale dusky.

Measurements, average of two individuals, as follows: Recently hatched larva, length of body 2.11 mm., width of first thoracic segment 0.35 mm., of cervical shield

[1] All descriptions and measurements, unless otherwise noted, are from living specimens.

0.25 mm., of abdominal segments 0.28 mm., width of head 0.35 mm. Just before first molt, length of body 4.02 mm., width of prothoracic segment 0.464 mm., width of abdominal segment 0.474 mm., length of cervical shield 0.310 mm., width 0.116 mm., width of head 0.35 mm., length of cephalic hairs 0.155 mm., abdominal hairs 0.080 mm.

SECOND INSTAR (immediately after first molt).—Head very pale, shining translucent, and with a very faint tint of raw sienna; no apparent reticulate markings such as appear in later instars; tips of mouth parts brownish to black. Ocelli black and arranged as in the first instar, but more prominent and not so closely placed. Cervical shield shining, translucent, and inconspicuous, and thoracic legs faintly dusky translucent. Ground color of anterior half of body pale green, of the posterior half whitish and with stripes as in preceding instar, but the darker lines more of a yellow-ocher color. Ventral surface whitish or greenish white. Pseudo and anal legs pale translucent. The fine hairs covering the body are whitish and placed on rather conspicuous black tubercles.

Just before the second molt, the head a shining, light raw umber; mouth parts darker. The ground color of the prothoracic segment whitish, due to the head of the third-instar larva, which is plainly visible through the translucent skin. Body gradually narrowing to anal segment. Otherwise similar in markings to the recently molted second-instar larva, except that the legs are slightly darker, and the lowest brown longitudinal line is paler, tending to yellowish orange.

Measurements, average of two individuals, as follows: Recently molted larva, length of body, 3.10 mm., width of prothoracic segment 0.531 mm., width of proanal segment 0.368 mm., width of head 0.58 mm., length of longest cephalic hairs 0.348 mm.

SECOND INSTAR (just before second molt).—Length of body 6.15 mm., width of prothoracic segment 0.71 mm., abdominal segments vary in width from 0.928 mm. for the first abdominal segment to 0.74 mm. for the proanal segment; width of head 0.56 mm.

THIRD INSTAR (just before third molt).—The head shows the reticulation or brownish mottlings illustrated by Forbes.[1] Prothoracic segment transparent, the pale reddish brown reticulations and ocelli on the head of the fourth-instar larva plainly visible beneath. Body markings as in preceding description, excepting that the second dorsal brown band has broken into two brown bands and one whitish band; the third brown band has a paler more or less conspicuous line along its median; the brown band at base of legs almost obliterated; the longitudinal stripes become very faint at the anterior and less so at posterior end. The general color of the body is greenish at anterior end and cream tinted posteriorly. Ventral surface greenish white. Body hairs whitish, except those on dorsum, which are blackish. First pair of abdominal legs now fully developed. Otherwise as in previous instars.

Measurements, average of two specimens, as follows: Just before third molt, length of body 10.35 mm., width of prothoracic segment 1.26 mm., of anal segment 1.05 mm., of head 0.95 mm.

FOURTH INSTAR (just after third molt).—Head pale umber with the reticulated markings of raw sienna, as in third instar, but more prominent. General color of body Nile green, paler posteriorly. The longitudinal stripes as in preceding instar, except that lines below the line of spiracles have become entirely obliterated, the color below the spiracles being whitish green to Nile green. The dorsal lines are inconspicuous or indistinct at their extremities. Otherwise as in preceding instars.

FOURTH INSTAR (just before the fourth molt).—Head pale translucent with reticulated areas of dusky brown, darker than earlier in instar. The prothoracic segment swollen, showing head of larva of fifth instar; a light raw sienna, the reticulated mark-

[1] Forbes, S. A. A monograph of insect injuries to Indian corn. Part II. 23d Rpt. State Ent. Ill., p. 84, fig. 63 b. 1905.

ings on it plainly visible. The longitudinal stripes arranged as in preceding description, the color of the lines becoming intermixed and less distinct; the white spiracular stripe just below the line of spiracles with an interrupted ocherous line, not observed in preceding instar. The general body color is pale greenish on thoracic segments, the remaining segments gradually changing to pale yellowish brown. Spiracles black, surrounded by rather conspicuous whitish areas, those on prothoracic and proanal segments largest.

Measurements, average of two individuals, just after third molt: Length of body 10.7 mm., width of prothoracic segment 1.47 mm., of anal segment 1.28 mm., of head 1.45 mm. Just before fourth molt: Length of body 15.0 mm., width of prothorax 1.86 mm., of anal segment 1.55 mm., of head 1.47 mm.

FIFTH INSTAR (just after fourth molt).—Head as described for previous instar. Ocelli become more distant with growth of head, the posterior one of the two near base of antenna reduced to an inconspicuous spot, much resembling a seta spot. The longitudinal stripes on abdomen as in preceding instar, except that just below the line of spiracles is a narrow white line followed by another rather narrow line of reddish burnt umber, the underside of body cream-colored or with faint greenish tint; dorsal surface appearing mottled with brownish markings on pale or cream-colored background. The median slit of spiracle is more conspicuous and shows as a paler whitish area. Otherwise as in preceding instar.

FIFTH INSTAR (nearly full-grown fifth-instar larva while feeding).—The entire body from dorsal view a dirty greenish color with pink tint, the former darkest near central portion of body and along median dorsal lines and the latter more prominent at the extremities, obliterated by the dull-green coloration of the dorsum. Below the line of spiracles is a pale pinkish green longitudinal line bordered on either side by a narrow whitish line, above and below which the more or less mottled dusky-green color predominates. Spiracles as before, but of a more velvety black. Legs as before, but the pseudolegs with dusky encircling bands at extreme base visible when legs are fully extended. Otherwise as previously described.

FIFTH INSTAR (just before fifth molt).—Head and prothoracic segments of same general appearance as in preceding instar just before molting. From a dorsal view the general color is pale yellowish green to cream, the longitudinal lines being very faint with no definite outline. The broad longitudinal dark stripe just above line of spiracles contrasts strongly with the whitish or cream-colored area below the spiracles. Other markings as in preceding instar.

Measurements, average of two individuals, just after fourth molt: Length of body 13.85 mm., width of prothoracic segment 2.13 mm., of anal segment 1.64 mm., of head 2.30 mm. Nearly full grown and while feeding: Length of body 20.7 mm., width of prothoracic segment 2.59 mm., of head 2.44 mm. Just before fifth molt: Length of body 20.55 mm., width of prothoracic segment 2.90 mm., of proanal segment 2.32 mm., of head 2.38 mm.

SIXTH INSTAR (about full grown, Pl. CVII, *D*, but still feeding).—Head reticulated and mottled as before, but median suture with border of dark raw umber. Cervical shield more prominent and shining, covering almost the entire dorsum of the prothoracic segment. General color from dorsal view dirty-pale brown, paler at posterior third. The general color varies in different individuals, some being very pale while others appear very dark and in some cases even almost black. At the beginning of this instar the general color was pale with a distinct pinkish tint and the median-dorsal area was dull green, the pinkish shades predominating at the posterior extremity. A median dorsal and two conspicuous white lines laterad on dorsum of prothorax, each of the latter bordered on its inner side by a narrow dark-brown area. These white lines are extensions of longitudinal lines extending the length of the body, but are much more prominent and distinct on the prothoracic segment. The median dorsal line or stripe dark brown, its median interrupted at

intervals by narrow white dashes, these the remnants of the white median line of previous instars; laterad to this median stripe is a pale line which becomes interfused with the former along its border; below is an interrupted dark-brown stripe followed by a white stripe, and this by a rather conspicuous dark-brown stripe of the same width as last and just above line of spiracles. Below the line of spiracles is a conspicuous yellowish or cream-colored stripe with a more or less pinkish tint, contrasting with the dark-brown line above and the dusky-brown area beneath. Ventral surface of body uniformly pale, but slightly brownish or dusky, the dorsum appearing mottled and the pinkish shades rather conspicuous. Body sparsely covered with very fine hairs placed on minute black tubercles. Spiracles as in preceding instar. Legs pale dusky at joints and at tips. Pseudolegs pale and when extended a conspicuous black band is visible on outer side and extending halfway round the legs at their base.

Measurements, average of two individuals, shortly after fifth molt: Length of body 24 mm., width of prothoracic segment 3.41 mm., of head 3.48 mm. Full grown: Length of body 35 mm., width of prothorax 3.8 mm., of widest abdominal segment 6.0 mm., of anal segment 3.5 mm., of head 3.4 mm.

Individual 75, which had an extra instar—that is, seven—agreed in general markings with the description for the fifth-instar larva given above. Measurements for this individual just before sixth molt as follows: Length of body 21.2 mm., width of prothoracic segment 2.9 mm., of widest abdominal segment 4.0 mm., of proanal segment 2.4 mm., of head 2.4 mm.

The head widths are apparently good and substantial characters for distinguishing larvæ of different instars, the width varying only slightly in different individuals and never varying in any instar for the same individual. In Table III are given the average head widths for each instar, the records here given showing only those measurements actually recorded in descriptive notes, although many other corroborative measurements were made at frequent intervals.

TABLE III.—*Width of head (in millimeters) of Cirphis unipuncta at different stages of growth*

| Number of individual in series. | First instar. | Second instar. | Third instar. | Fourth instar. | Fifth instar. | Sixth instar. |
|---|---|---|---|---|---|---|
| 11 | 0.348 | | | | | |
| (?) | .348 | | | | | |
| 45 | | 0.581 | | | | |
| 42 | | .581 | | | | |
| 47 | | .581 | | | | |
| 48 | | .542 | | | | |
| 146 | | .581 | | | | |
| 152 | | .581 | | | | |
| 39 | | | 0.968 | | | |
| 44 | | | .929 | | | |
| 9 | | | | 1.432 | | |
| 38 | | | | 1.471 | | |
| 22 | | | | 1.471 | | |
| 30 | | | | | 2.326 | |
| 36 | | | | | 2.284 | |
| 144 | | | | | 2.361 | |
| 153 | | | | | 2.400 | |
| 133 | | | | | 2.439 | |
| 61 | | | | | | 3.484 |
| 123 | | | | | | 3.400 |
| Average | .348 | .574 | .948 | 1.458 | 2.362 | 3.442 |

## THE PUPA

When fully colored of a shining mahogany brown, and comparatively smooth. In general appearance not unlike other noctuid pupæ. Dorsum of thorax with noticeable transverse wrinkles, the mesothorax with a more or less distinct smooth line along the median dorsum. Bases of first four abdominal segments on dorsum and of fifth, sixth, and seventh segments on venter rather indistinctly and sparsely punctured; the fifth, sixth, and seventh segments with a ridge near the anterior border on dorsum which reaches nearly to spiracles on each side, the posterior margin of this

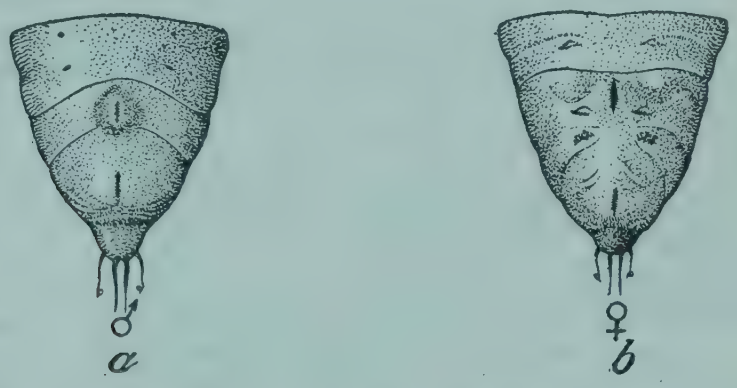


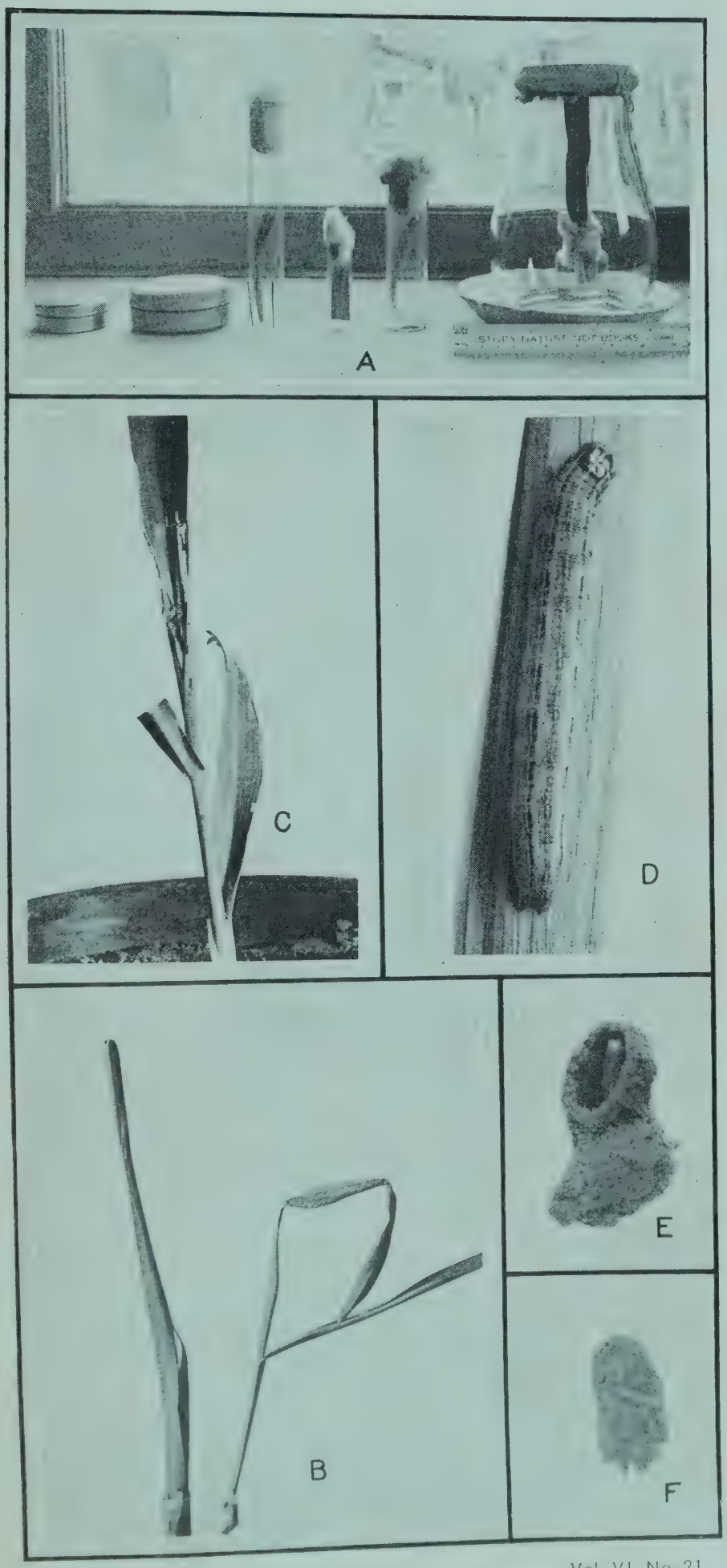

FIG. 2.—Posterior extremity of male and female pupa: *a*, Male; *b*, female.

ridge being denticulate or deeply crenulate, the teeth directed caudad and most prominent at median dorsum and gradually becoming less distinct laterally. The male and female pupæ are readily separated by a comparison of the accompanying illustrations (fig. 2).

Measurements: Length 19.3 mm.; width at tip of wings of male 6.2 mm., of female 7.0 mm. Length of the two prominent robust spines at tip of body in male 0.55 mm., in female 0.7 mm.; a more slender and more or less hooklike spine on each side of the larger one is shorter.

## PLATE CVII

*A*, Cages for rearing *Cirphis unipuncta; B*, leaves glued together after the eggs have been deposited; *C*, characteristic leaves partly eaten by first-instar larvæ; *D*, full-grown larva; *E* and *F*, characteristic pupal cells.


A
C
D
B
E
F

# INFECTION OF TIMOTHY BY PUCCINIA GRAMINIS

By E. C. Stakman, *Head of the Section of Plant Pathology*, and F. J. Piemeisel, *Research Assistant, Division of Plant Pathology and Botany, Department of Agriculture, University of Minnesota* [1]

It has been shown a number of times that *Puccinia phleipratensis* Eriks. and Henn. can infect oats (*Avena sativa*) and rye (*Secale cereale*), and it has also been shown recently that it can infect barley (*Hordeum vulgare;* (8, p. 213).[2] Inoculation of timothy (*Phleum pratense*) with *Puccinia graminis* was reported by Eriksson (2, p. 71), Johnson (3, p. 9), Mercer (6, p. 22), Stakman and Jensen (8, p. 213), and others as giving only negative results. Carleton, however (1, p. 62), succeeded in infecting *Phleum asperum* with *P. graminis avenae.*

The timothy-rust problem offers a good field for investigating the possible origin and developmental tendencies of biologic forms. The rust can infect oats, rye, barley, and a number of wild grasses; but morphologically it differs from *P. graminis*, and its ability to infect barberry (*Berberis vulgaris*) regularly is still a matter of doubt (3, p. 11). From its close similarity to *P. graminis avenae*, however, it seems reasonable to suppose that it may possibly have developed from some form of *P. graminis*. Since *P. phleipratensis* resembles *P. graminis avenae* parasitically more closely than any other biologic form of *P. graminis*, it would seem that infection of timothy with *P. graminis avenae* might be possible. For this reason the writers made a very large number of inoculations on a number of strains of timothy.

All inoculations were made on seedlings from 3 weeks to 3 months old. The leaves were first thoroughly moistened and then inoculated heavily with urediniospores of *P. graminis avenae* originally isolated from *Dactylis glomerata* and then kept on oats in the greenhouse for 14 months, having been transferred 30 times during that period. The rust had been used extensively in a large number of inoculation experiments, and the fact that it was a normal strain of *P. graminis avenae* had been well established. After inoculation the pots containing the seedlings were put in pans containing a small amount of water and were then kept covered with bell jars for 48 hours. At the end of that time they were removed and kept on an ordinary greenhouse bench. Inoculations were made with other biologic forms of *P. graminis* also; none of these, however, resulted in infection, therefore serving as checks. The "ordinary" timothy seed used was obtained from the Minnesota

[1] In cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] Reference is made by number to "Literature cited," p. 816.

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Seed Laboratory, and was selected from seed trade samples. The Cornell and Minnesota selections or strains were obtained from the Section of Plant Breeding, Division of Agronomy and Farm Management, Minnesota Experiment Station. A summary of the results of inoculations is given in Table I.

TABLE I.—*Results of inoculations with Puccinia graminis on Phleum pratense*

| Date of inoculation. | Source of uredinio-spores. | Strain of *Phleum pratense* inoculated. | Number of leaves inoculated. | Number of leaves infected. | Date of inoculation. | Source of uredinio-spores. | Strain of *Phleum pratense* inoculated. | Number of leaves inoculated. | Number of leaves infected. |
|---|---|---|---|---|---|---|---|---|---|
| 1915. | | | | | 1916. | | | | |
| Dec. 8 | *P. graminis avenae* from *Avena sativa.* | Ordinary timothy. | 48 | 0 | Mar. 23 | *P. graminis avenae* from *Avena sativa.* | Minnesota 79 | 170 | 8 |
| 1916. | | | | | Mar. 3 | *P. graminis tritici* from *Hordeum vulgare.* | Ordinary timothy. | 28 | 0 |
| Jan. 25 | .....do..... | .....do..... | 50 | 5 | | | | | |
| Feb. 7 | .....do..... | .....do..... | 76 | 2 | | | | | |
| Feb. 18 | .....do..... | .....do..... | 234 | 3 | | | | | |
| Mar. 2 | .....do..... | Cornell 1671. | 248 | 7 | Mar. 16 | .....do..... | .....do..... | 360 | 0 |
| Do.... | .....do..... | Cornell 1743. | 140 | 6 | Mar. 30 | .....do..... | .....do..... | 100 | 0 |
| Do.... | .....do..... | Cornell 1611. | 160 | 4 | Mar. 4 | .....do..... | .....do..... | 30 | 0 |
| Do.... | .....do..... | Cornell 1715. | 120 | 0 | Do.... | .....do..... | Cornell 1687. | 88 | 0 |
| Do.... | .....do..... | Cornell 1630. | 96 | 0 | Do.... | .....do..... | Minnesota 63 | 168 | 0 |
| Do.... | .....do..... | Cornell 1676. | 134 | 1 | Apr. 6 | *P. graminis secalis* from *Secale cereale.* | Cornell 1715. | 78 | 0 |
| Do.... | .....do..... | Minnesota 78 | 196 | 5 | | | | | |
| Do.... | .....do..... | Cornell 3230. | 170 | 1 | | | | | |
| Do.... | .....do..... | Cornell 1687. | 100 | 4 | | | | | |
| Do.... | .....do..... | Cornell 1777. | 94 | 0 | | | | | |
| Do.... | .....do..... | Cornell 1620. | 240 | 1 | Do.... | .....do..... | Cornell 1635. | 60 | 0 |
| Do.... | .....do..... | Minnesota 50 | 180 | 4 | Do.... | .....do..... | Cornell 1630. | 66 | 0 |
| Do.... | .....do..... | Minnesota 70 | 150 | 0 | Do.... | .....do..... | Cornell 1611. | 60 | 0 |
| Do.... | .....do..... | Minnesota 79 | 200 | 0 | Mar. 17 | *P. graminis secalis* from *Hordeum vulgare.* | Cornell 3230 | 72 | 0 |
| Do.... | .....do..... | Minnesota 63 (G. Bros. 2501). | 186 | 6 | | | | | |
| Do.... | | | | | | | | | |
| Do.... | .....do..... | Minnesota 63 (G. Bros. 3801). | 140 | 0 | Mar. 8 | *P. graminis secalis* from *Elymus virginicus.* | Cornell 1676 | 50 | 0 |
| Mar. 23 | .....do..... | Minnesota 77 | 140 | 0 | | | | | |
| | | | | | Do.... | .....do..... | Cornell 1687. | 68 | 0 |

It will thus be seen that successful infection resulted in 14 of the 22 trials with *P. graminis avenae* and it occurred on at least 11 different selected strains which when grown in the greenhouse varied considerably in type and vigor. Unpublished results obtained by Mr. M. N. Levine, a graduate student in the University of Minnesota, corroborate the work done by the writers. Mr. Levine's inoculations were made with another strain of *P. graminis avenae*, and, although there is probably little or no difference between one strain of this rust and another, it is interesting to know that the results obtained are not due to the peculiarities of the particular rust strain used. Inoculations on oats with the spores produced on timothy resulted in the formation of typical pustules in about eight days. None of the 774 timothy leaves inoculated with *P. graminis tritici* produced pustules, and none of the 454 inoculated with *P. graminis secalis* became infected, although the writers are not convinced that these transfers are impossible.

It is quite evident both from the percentage of successful infections and from the character of the infection that timothy can not be considered a congenial host for *P. graminis avenae*. The total number of leaves inoculated was 3,270 and only 57 became infected, only 1.47 per cent. The pustules were always small, ranging in size from mere dots to pustules 0.3 mm. in diameter. On the older leaves they were often surrounded by a small dead area, indicating a certain degree of hypersensitiveness, while on younger leaves they often appeared to develop quite normally except in size. Four or five pustules sometimes developed on the same leaf, giving the appearance of fairly successful infection. The incubation period varied from 8 to 12 days. The spores were considerably smaller in size than those of *P. graminis avenae*, but they were larger than those of *P. phleipratensis*. The spores of *P. graminis avenae* are also reduced in size on barley; the character of infection is somewhat the same as that on timothy and the spores become almost identical in size. Comparative measurements of spore lengths are given in Table II.

TABLE II.—*Length of urediniospores of P. graminis avenae and P. phleipratensis*

| Rust organism. | Host on which measured. | Length limits. | Mode. |
|---|---|---|---|
| | | μ | μ |
| *P. graminis avenae* | *Avena sativa* | 24 to 35.52 | 29.44 |
| Do | *Phleum pratense* | 20.16 to 32.64 | 25.60 |
| Do | *Hordeum vulgare* | 20.80 to 32.96 | 25.60 |
| *P. phleipratensis* | *Phleum pratense* | 16 to 28.80 | 21.76 |

Although the size of the spores is decreased on timothy, it becomes normal the first generation when the rust is transferred back to oats. The decrease in size is probably to be regarded only as a stunting due to unfavorable environment, since it has been previously shown that spores of *P. graminis* produced on an uncongenial host tend to become smaller than on a congenial host (7, p. 31). The color of the spores remains constant on oats and timothy and they can thus be distinguished very easily from spores of *P. phleipratensis*. Spores of *P. graminis avenae* are a bright cadmium-yellow in color, while those of *P. phleipratensis* are much duller, sometimes almost gray.

The fact that *P. graminis* can infect timothy raises the question as to whether *P. phleipratensis* may not have developed from some biologic form of this rust. Only speculation is possible at the present time, and a discussion of the possibilities is therefore probably useless. Nevertheless it is significant that *P. graminis avenae*, which now seems a possible source of the rust, produces urediniospores of very different shapes and sizes on the same plant and in the same pustules, thus conceivably indicating a tendency toward instability. The rust also has a wide range of hosts, in that while occurring commonly on one cereal, oats, and being

capable of infecting two others, barley and rye, it is also capable of infecting many wild grasses in this country and Europe. Until further, more extensive attempts are made to infect barberries with teliospores of *P. phleipratensis* and until the possibilities of developing experimentally a strain of *P. graminis* on timothy have been exhausted, work is more desirable than words, but the fact that *P. phleipratensis* can infect three of the cereals and a number of grasses and that timothy can be infected by *P. graminis avenae* may possibly indicate that timothy rust, as Kern (4, 5) has previously suggested, may not be so far removed from *P. graminis* as has sometimes been supposed.

## SUMMARY

(1) It has been possible by means of artificial inoculations to infect various strains of timothy with *Puccinia graminis avenae*.

(2) Timothy exerted an appreciable effect on the morphology of spores of *P. graminis avenae*, reducing them considerably in size. Practically identical results were obtained by transferring the rust to barley.

(3) The rust was subnormal in vigor on timothy, the pustules always remaining small.

(4) The facts recorded in this paper are suggestive of the possible origin of *P. phleipratensis*.

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# CONTROL OF THE POWDERY DRYROT OF WESTERN POTATOES CAUSED BY FUSARIUM TRICHOTHECIOIDES

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## INTRODUCTION

Wherever potatoes (*Solanum tuberosum*) are grown, storage-rots occur. These rots are in the majority of cases caused by wound parasites which attack the potato tubers through bruises in the skin occasioned by the handling of the potato crop in harvesting. A type of storage dryrot known as "powdery dryrot" and ascribed to the parasite *Fusarium trichothecioides* Wollenw. is apparently restricted to the arid and semiarid sections of the western part of the United States. Undoubtedly rots due to other causes also occur, but powdery dryrot is the only storage-rot causing enough damage to be of any great economic importance in the irrigated West. It would be difficult to arrive at any definite statement of the losses entailed by this disease, but it is known that they have been enormous. In several cellars visited, the writer estimated the losses caused by partial and total decay of the tubers to be from 30 to 50 per cent. Reports from farmers show that in some cases the losses have been much greater. This storage dryrot may be described as an external dryrot proceeding from bruises in the skin of the tuber. The decayed portion usually presents a wrinkled, sunken appearance, and in advanced stages may show a pinkish white growth of the fungus (Pl. CVIII, fig. 1, 2). The decayed tissue presents various shades of color from nearly black to light brown, the most characteristic color being sepia brown. Internal cavities partially filled with the mycelium and spores of the fungus are frequently found in decayed tubers (Pl. CVIII, fig. 3).

The first description of this disease was made in 1912 by Jamieson and Wollenweber,[1] who demonstrated that the rot was caused by a species of Fusarium which they called "*Fusarium trichothecioides* Wollenw." One year later Wilcox, Link, and Pool [2] described a dryrot of potato tubers in Nebraska, ascribing it to a species of Fusarium which they called "*Fusarium tuberivorum.*" This fungus has since been demonstrated by Wollen-

[1] Jamieson, Clara O., and Wollenweber, H. W. An external dry rot of potato tubers caused by Fusarium trichothecioides Wollenw. *In* Jour. Wash. Acad. Sci., v. 2, no. 6, p. 146–152, 1 fig. 1912.

[2] Wilcox, E. M., Link, G. K. K., and Pool, Venus W. A dry rot of the Irish potato tuber. Nebr. Agr. Exp. Sta. Research Bul. 1, 88 p., 15 fig., 28 pl. (1 col.). 1913. Bibliography, p. 85–88.

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weber [1] and by Carpenter [2] to be identical with *F. trichothecioides* Wollenw. Wilcox, Link, and Pool [3] found that their fungus was incapable of attacking the tubers through the eyes or lenticels and that it was incapable of attacking the growing plants. Jamieson and Wollenweber,[3] however, working with *F. trichothecioides* obtained from western potatoes, found that *F. trichothecioides* was capable of attacking the growing plant, and they also obtained infections through the unbroken skin of the tuber by rubbing the inoculum over the surface. Their results were obtained under the extremely humid conditions of the Department greenhouses at Washington, D. C.

Working with the same fungus, the writer was unable under the western field or laboratory conditions to produce infection through the unbroken skin of the potato tuber or to produce an infection in any part of a growing potato plant. His results agree in the main with those obtained by Wilcox, Link, and Pool,[4] thus further establishing the identity of *F. trichothecioides* with the so-called *F. tuberivorum*.

Preliminary work on this potato-tuber disease was begun in 1912, when the author was connected with the Agricultural Experiment Station of the University of Idaho. During the fall of 1912 and the spring of 1913 potato shippers reported heavy losses in carload lots of potatoes en route from Idaho and Utah to eastern and southern markets. Examination of infected tubers from such cars invariably revealed the presence of *F. trichothecioides*. In the fall of 1913 the writer was enabled to begin a study of storage conditions of potatoes. This study was continued up to the spring of 1916. It is safe to say that powdery dryrot can be found in every potato storage cellar in the areas covered by the author's investigations. However, when storage conditions were found to be good, losses were being reduced to a minimum.

During the whole course of the investigations, experiments leading to a further knowledge of the relationship of the fungus to the disease, as well as practical experiments leading to its control, were carried on. These experiments were conducted in part in the laboratories in Washington, D. C., and in part in the field, laboratory, and storage cellar of the Jerome Experiment Station, Jerome, Idaho. The work was further supplemented by the planting of seed plots in various places in southern Idaho. The results of these experiments, as set forth in this paper, are believed to be of fundamental scientific importance, since they throw more light on the relationship of the fungus to the disease and demonstrate a fairly successful method of control.

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[1] Wollenweber, H. W. Ramularia, Mycosphaerella, Nectria, Calonectria. Eine morphologisch pathologische Studie zur Abgrenzung von pilzgruppen mit cylindrischen und sichelförmigen Konidienformen. *In* Phytopathology, v. 3, no. 4, p. 206. 1913.

[2] Carpenter, C. W. Some potato tuber-rots caused by species of Fusarium. *In* Jour. Agr. Research, v. 5, no. 5, p. 183–210, pl. A–B (col.), 14–19. 1915. Literature cited, p. 208–209.

[3] Jamieson, Clara O., and Wollenweber. Op. cit.

[4] Wilcox, E. M., Link, G. K. K., and Pool, Venus W. Op. cit.

## PARASITISM OF FUSARIUM TRICHOTHECIOIDES

To determine the parasitism of *F. trichothecioides*, several attempts were made to induce infection in various parts of growing plants and in mature tubers.

1. In the fall of 1913 half-bushel lots of unbruised tubers were obtained of each of the following varieties: Burbank, Idaho Rural, Early Rose, Peoples, Improved Peachblow, Netted Gem, and Pearl. All of the tubers selected were free from any external evidence of disease and were disinfected by dipping in a solution of formaldehyde (1:240). Each tuber in one half-bushel lot of each variety was bruised with a sterile knife and the bruised surface dipped in a suspension of the spores of the fungus. Each tuber in another half-bushel lot of each variety was first carefully examined to make sure that its skin was wholly sound and was then dipped in a suspension of the spores of the fungus. Checks of the same quantity of tubers of each variety were prepared in the same manner, except that the tubers, whether sound or bruised, were dipped in sterile water. Each lot was then placed in a sterilized canvas sack. To insure a high degree of humidity each sack was sprayed with sterile water. The sacks were then stored in one corner of the cellar and covered with canvas. The tubers were not examined until the following May, or about seven months after having been placed in storage. At the time of examination all tubers had sprouted, showing that temperature conditions, at least during the latter part of the storage period, had been ideal for the development of the rot. Every inoculated, bruised tuber showed infection, each bruised, inoculated tuber being from one-eighth to three-fourths decayed. None of the inoculated sound tubers showed any infection. In the checks there was a slight amount of decay in many of the bruised tubers, though they had not been inoculated; but all of the sound tubers of the checks remained sound throughout the storage period.

2. In the fall of 1914 further attempts were made to infect potato tubers with *F. trichothecioides* through the unbroken skin. The following varieties were employed: Improved Peachblow, Idaho Rural, Netted Gem, Peoples, and Pearl. Fifty sound tubers of each variety were first disinfected in a formaldehyde solution (1:240), dried, and then dipped in a spore suspension of the fungus. Fifty tubers of each variety were disinfected in the same manner, bruised with a sterile knife, and dipped in a suspension of the spores of the fungus. Each lot of tubers was then placed in a disinfected canvas sack. The potatoes were first stored in the laboratory culture room, where the temperature was very favorable to the development of the decay. A high humidity was maintained in the culture room by spraying the walls with sterile water. After a month the potatoes were removed from the culture room to the potato storage cellar, where they remained until spring. An examination of

the potatoes was made in April, 1915. None of the unbruised tubers showed any signs of infection, but infection was present in each of the bruised tubers.

3. In 1914, attempts to artificially infect growing potato plants with *F. trichothecioides* were made as follows:

*a*. One hundred apparently healthy Idaho Rural plants were selected, and the stem of each was punctured at the crown with a needle inoculated with the spores of the fungus. As a check, twenty-five apparently healthy plants of the same variety were selected and their stems punctured at the crown with a sterile needle.

*b*. One hundred apparently healthy Idaho Rural plants were selected. The soil was removed to expose one tuber under each plant. One tuber under each plant was punctured with a needle inoculated with the spores of the fungus. As a check, twenty-five apparently healthy plants of the same variety were selected and the soil removed to expose one tuber under each plant, which was then punctured with a sterile needle.

*c*. One hundred apparently healthy Idaho Rural plants were selected and the soil removed to expose one tuber under each plant. The stolon of one tuber under each plant was then punctured with a needle inoculated with the spores of the fungus. As a check, twenty-five apparently healthy plants of the same variety were selected and the soil removed to expose one tuber under each plant. One tuber stolon under each plant was then punctured with a sterile needle.

An examination was made one month later. No evidence of infection could be found in the proximity of the punctures in the stems, the tubers, or the tuber stolons. The punctures made were so large that they could be seen easily in each case, but apparently they had healed over. The checks presented the same appearance.

As the foregoing inoculations of growing plants had been made rather late in the season (August 21), it was thought that the failure to develop any infection might have been due to the late date on which the inoculations were made. Therefore the attempts were repeated in 1915 as follows:

1. Fifty Netted Gem tubers which had been inoculated with *F. trichothecioides* were kept for several days in moist chambers at temperatures favorable for the development of the fungus. On June 4, when the decay was well advanced, the fifty tubers were planted in a Station plot in an attempt to infect the growing plants through the seed pieces. A similar number of hills of the Netted Gem variety were planted with disease-free seed pieces as a check. The plants were examined from time to time during the season, cultures being made whenever any evidence of disease appeared, but *F. trichothecioides* was never obtained. The plot was dug on September 15, when all stems and tubers were examined for evidence of disease. There was no evidence of decay in the harvested tubers, and the stems of the plants were usually white and clean. Six

plants out of the fifty which resulted from the planting of the inoculated seed pieces showed vascular infection, but *F. trichothecioides* could not be recovered.

2. Twenty-five Idaho Rural tubers, first disinfected by dipping in formaldehyde, were placed in moist chambers and allowed to develop sprouts. On July 10, when the sprouts were from one-eighth to one-half inch long, they were sprayed with a spore suspension of the fungus. As a check, twenty-five Idaho Rural tubers were treated in the same manner, but the sprouts were sprayed with sterile water. After a little more than a month each sprout was carefully examined. No evidence of infection was found either in the sprouts sprayed with the spore suspension or in the checks.

3. On July 11 further attempts to infect growing potato plants were made as follows:

*a.* Ten apparently healthy Idaho Rural plants were selected. The soil was removed to expose as many of the tubers as possible without disturbing their position. In all, twenty-five tubers were uncovered and punctured with a needle inoculated with the spores of the fungus, after which the soil was replaced. As a check, a similar number of plants of the same variety were selected and twenty-five tubers punctured with a sterile needle. This experiment was duplicated with Netted Gems.

*b.* Ten apparently healthy Idaho Rural plants were selected. The soil was removed to expose as many of the tubers as possible without disturbing their position. Twenty-five tubers thus uncovered were sprayed with a suspension of the spores of the fungus, after which the soil was replaced. To prevent the rapid drying off of the sprayed tubers, the soil when replaced was moistened. As a check, ten other apparently healthy Idaho Rural plants were selected and twenty-five tubers sprayed with sterile water. The soil was moistened upon being replaced. This experiment was duplicated with Netted Gems.

*c.* Ten apparently healthy Idaho Rural plants were selected. The soil was removed to expose as many of the tubers with their stolons as possible without disturbing their position. The stolons of twenty-five tubers thus uncovered were then punctured with a needle inoculated with the spores of the fungus, after which the soil was replaced, care being exercised to place moist soil next to the inoculations. As a check, ten other plants of the same variety were selected and twenty-five tuber stolons punctured with a sterile needle, after which the soil was replaced. This experiment was duplicated with Netted Gems.

*d.* Ten apparently healthy Idaho Rural plants were selected and the stem of each plant punctured at the crown with a needle inoculated with the spores of the fungus. As a check the stems of ten plants were punctured with a sterile needle. This experiment was duplicated with Netted Gems.

*e*. The leaves of ten apparently healthy Idaho Rural plants were sprayed with a spore suspension of the fungus. As a check, the leaves of ten apparently healthy plants were sprayed with sterile water. This experiment was duplicated with Netted Gems.

In the fall a careful examination was made of each plant and tuber. Not the slightest trace of infection that could be ascribed to *F. trichothecioides* could be found, though cultures were made from suspicious-looking stem lesions and tuber discolorations. The punctures in the tubers and stolons had healed over, leaving only the slightest scars as evidence. The punctures in the stems could be found by very careful scrutiny, but were entirely healed over. There was neither internal nor external evidence of disease in the neighborhood of the punctures, whether in stems, tubers, or tuber stolons. No disease appeared in the foliage or stems as a result of spraying with the spore suspension.

The results of the attempts to induce infection in growing potato plants were such as might have been expected after several years' search in commercial fields for evidence of disease which could be attributed to this organism. Several hundred cultures have been made from diseased parts of growing potato plants. Out of these attempts, *F. trichothecioides* has been obtained but 13 times, twice from Netted Gem tubers infected with jelly-end rot and 11 times from potato stems infected with footrot. In the footrot cultures, *F. trichothecioides* was associated with other species of Fusarium, including *F. radicicola* and *F. oxysporum*, as well as other fungi. It is not likely that *F. trichothecioides* attacked the growing stem, but rather it is probable that it entered as a secondary organism after the attacks of other fungi or bacteria. The writer has shown in another paper [1] that jelly-end rot does not develop at temperatures below 10° C.; therefore *F. trichothecioides* is eliminated as one of the contributing causes of this fieldrot, since, if *F. trichothecioides* were generally present in tubers infected with jelly-end rot, such tubers when placed in storage would continue to decay at temperatures as low as 4° (see pages 825 to 827). *F. trichothecioides* has never been obtained from any other fieldrot. In commercial storage cellars, unbruised tubers have never been found infected by *F. trichothecioides;* on the other hand, the majority of bruised tubers in storage show more or less decay from this cause.

## EFFECT OF PLANTING SEED INFECTED BY DRYROT

Poor stands of potatoes have been observed from year to year in many potato fields in southern Idaho. In many cases it was impossible to say whether the poor stand was due to irregularity in planting or to poor seed. In some cases, however, the only explanation that could be made

[1] Pratt, O. A. A western fieldrot of the Irish potato tuber caused by Fusarium radicicola. *In* Jour. Agr. Research, v. 6, no. 9, p. 297–310, pl. 34–37. 1916.

was the known fact that seed infected with dryrot had been planted. To determine the effect on the stand of planting seed infected with dryrot, several plots of potatoes were planted as follows:

Plot 1.—Each seed piece showed at least one healthy eye, but was almost wholly decayed. The variety planted was Idaho Rural.

Plot 2.—Each seed piece showed a pocket of dryrot at least half an inch in diameter and about as deep as wide. The variety planted was Idaho Rural.

Plot 3.—This plot was planted with seed of the same character as that used in plot 1, except that the variety was Netted Gem.

Plot 4.—This plot was as nearly as possible a duplicate of plot 2, except that the variety planted was Netted Gem.

Two check plots were also planted, one of Netted Gem and one of Idaho Rurals. In each of the check plots only seed entirely free from disease was used. The plots were planted on the grounds of the experiment station at Jerome, Idaho. Table I shows the stand which resulted.

TABLE I.—*Percentage of stand of potatoes in plots infected with powdery dryrot*

| Plot No. | Variety. | Percentage of stand. | |
|---|---|---|---|
| | | Four weeks after planting. | Six weeks after planting. |
| 1 | Idaho Rural | 72 | 82 |
| 2 | do | 98 | 100 |
| 3 | Netted Gem | 70 | 85 |
| 4 | do | 99 | 100 |
| Check | do | 100 | 100 |
| Do | Idaho Rural | 100 | 100 |

In plots 1 and 3, in which the seed planted was nearly totally decayed, the stand never exceeded 82 per cent of the Idaho Rural nor 85 per cent of the Netted Gem. The plants in these two plots were much slower in coming up than those in the check plots or in plots 2 and 4. One month after planting, all the seed in the check plots had produced plants larger and stronger than those in plots 1 and 3, but no difference was observed between the plants in the check plots and those in plots 2 and 4. Although the stand in plots 2 and 4 was not quite perfect at the end of the first month, the stragglers soon appeared, and a perfect stand resulted. The results of these experiments specifically agreed with the observations in commercial fields. It is believed that had the wet weather of the early spring continued throughout the month of June, a much smaller percentage of the seed would have produced plants. The plots were carefully watched throughout the growing season; but after the plants had thoroughly established themselves, there was little or no difference between the plants in the diseased plots and those in the

check plots. The plants in plots 1 and 3 eventually became as strong and vigorous as those in plots 2 and 4 and in the check plots. At harvest time 100 hills from each plot were dug and the tubers carefully examined for the evidence of disease. Table II shows the percentage of disease present in the tubers at harvest time.

TABLE II.—*Percentage of disease present in potato tubers at harvest time*

| Plot No. | Variety. | Scab. | Rhizoctonia scab. | Vascular infection. | Powdery dryrot. |
|---|---|---|---|---|---|
| 1 | Idaho Rural | 2 | 0 | 36 | 0 |
| 2 | do | 0 | 0 | 38 | 0 |
| 3 | Netted Gem | 0 | 0 | 32 | 0 |
| 4 | do | 0 | 0 | 33 | 0 |
| Check | do | 0 | 0 | 21 | 0 |
| Check | Idaho Rural | 0 | 0 | 59 | 0 |

It is evident from the results that dryrot infection in the seed does not in any way influence the amount of disease in the product. No dryrot appeared in any of the plots at harvest time. The percentage of vascular infection was higher in the case of one of the check plots and lower in the other than in the diseased seed plots. A large number of cultures were made from the discolored vascular tissues of the tubers from all of the plots, but the fungus *F. trichothecioides* was never once obtained.

## SOURCE OF THE ORGANISM CAUSING POWDERY DRYROT

It was evident that the organism causing the decay must be present in the soil particles clinging to the surface of the tubers when harvested, but whether *F. trichothecioides* was present in the soil prior to the planting of the potatoes or was introduced with the seed was not known. It was thought that the latter might be the case. Accordingly plots of potatoes in which all the seed was entirely free from disease and had been disinfected for 1½ hours in a solution of mercuric chlorid (1:1,000) were planted on both raw desertland and lands previously in alfalfa. Check plots were also planted in which each seed piece was well infected with the rot.

At harvest time samples of potatoes from each of the plots were placed in sterilized tin boxes and put in storage at temperatures favorable for the development of the rot. Each tin box used in the experiment was first wrapped in heavy paper and sterilized for three hours in the oven at a temperature of 160° C. To secure the samples of potatoes, the sterile boxes were taken to the field and a hill or more of potatoes dug with a trowel which had first been sterilized. The tubers were then bruised with the same trowel, the box opened, and the potatoes put into the box with a little of the moist soil in which the tubers had been growing, in order to insure proper moisture conditions within the box. The box was

then closed, wrapped, and stored. Eight of these samples were obtained: Two of Netted Gem and one of Idaho Rural from desert land plots, and one of the Netted Gem and two of Idaho Rural from alfalfa-land plots. The remaining two samples were taken from the check plots which were planted on alfalfa land. One was a sample of the Netted Gem variety, and the other was an Idaho Rural. Two months after storing the samples, the boxes were opened and the tubers examined. Every tuber in each of the eight boxes showed at least slight signs of decay, and some showed deep infection pockets of dryrot.

Isolations were made from the decayed portions of the tubers and the presence of the organism determined. Not a single culture gave negative results. It is apparent, therefore, that *F. trichothecioides* is at the present time well distributed in desert soils, as well as in those previously in cultivation, and is not necessarily introduced on the seed.

## RELATIONSHIP OF TEMPERATURE TO THE DEVELOPMENT OF POWDERY DRYROT

The experiments to determine the relationship of temperature to the development of powdery dryrot were carried on in the laboratories in Washington and in the cold-storage rooms of a Washington cold-storage plant. In these experiments potatoes of the following varieties were used: Idaho Rural, Netted Gem, Peoples, Pearl, Burbank, and Improved Peachblow. Three different experiments were undertaken.

1. Tubers of each of the above varieties were first washed and disinfected by fumigating with formaldehyde gas. Two methods of inoculation were employed. The first method consisted in cutting off the stem end of the tuber and dipping the cut surface into a spore suspension of *F. trichothecioides*. The second method consisted in inoculating the tubers by puncturing the skin with a needle inoculated with the spores of the fungus. In both cases the inoculated tubers were wrapped separately in sterile paper. Tubers inoculated by each of these methods were placed in the incubators and in the incubator room. Checks were prepared in the same manner except that the tubers in one case were dipped in sterile water and in the other case were punctured with a sterile needle.

2. In the second experiment sterile blocks were cut from tubers from each of the varieties named. These sterile blocks were placed in sterile culture tubes and allowed to incubate for several days in order to insure their sterility, after which they were inoculated with the fungus and placed in the incubators and in the incubator room.

3. In the third experiment half-bushel lots of each of the varieties above named were first washed and disinfected by fumigating with formaldehyde gas. Each tuber was then cut across the stem end and the cut surface dipped in a spore suspension of the fungus, after which they

were wrapped separately in sterile paper. Each half-bushel lot was then placed in a tin box which had first been sterilized. Half-bushel lots of each variety thus prepared were placed in cold storage at temperatures of 0° and 1.1° C. Half-bushel lots of each variety were prepared in the same manner and placed in the incubator room as a check. Check lots in which the tubers were treated in the same manner but not inoculated were also placed in the incubator room and in cold storage at temperatures of 0° and 1.1° C. Table III gives the results of these tuber inoculations under the different storage conditions, showing the temperatures of the incubator chambers, the incubator room, the rooms in the cold-storage plant during the period of storage, and the condition of the inoculated tubers at the end of the storage period. In Table III the incubator chambers are designated by numbers 1 to 10 and the cold-storage rooms as A and B. All of the uninoculated checks remained sound.

TABLE III.—*Results of potato-tuber inoculations under different storage conditions*

| Incubator chamber No. | Temperatures during period of storage. | | | Condition of inoculated tubers at termination of storage period. |
|---|---|---|---|---|
| | Minimum. | Maximum. | Average. | |
| | ° C. | ° C. | ° C. | |
| 1 | 0.2 | 2.1 | 0.8 | Sound. |
| 2 | 4.0 | 7.3 | 4.2 | Very slight decay. |
| 3 | 4.2 | 10.2 | 7.6 | One-third to two-thirds decayed. |
| 4 | 6.3 | 12.5 | 8.9 | Nearly total decay. |
| 5 | 8.9 | 14.4 | 12.0 | Total decay. |
| 6 | 10.0 | 17.0 | 14.7 | Do. |
| 7 | 11.0 | 19.9 | 17.0 | Do. |
| 8 | 14.0 | 21.8 | 18.0 | Do. |
| 9 | 15.0 | 25.1 | 19.0 | Do. |
| 10 | 13.8 | 22.5 | 19.9 | Do. |
| Incubator room | 19.0 | 26.5 | 25.0 | Do. |
| A | 0 | 0 | 0 | Sound. |
| B | 1.1 | 1.1 | 1.1 | Do. |

It is evident from the results obtained that powdery dryrot will not develop at temperatures below 2° C. At temperatures ranging from 2° to 4° (35° to 40° F.) the amount of decay will be slight, especially if the storage rooms are kept fairly dry and well ventilated.

## INFLUENCE OF HUMIDITY ON THE DEVELOPMENT OF POWDERY DRYROT IN STORAGE

It has often been observed in storage cellars which were comparatively dry and well ventilated that the losses from powdery dryrot were much less than in damp, poorly ventilated cellars. The writer has been in cellars where practically every bruised tuber was from one-third to nearly totally decayed. Such cellars have invariably been exceedingly damp

and poorly ventilated. He has been in other cellars where the bruised tubers showed only an incipient rot, the decay usually extending inward from the bruised surface for less than one-fourth of an inch. Such cellars have invariably been very dry and well ventilated. It is to be regretted that there has been no opportunity to obtain the percentages of atmospheric humidity most favorable to the development of the rot. However, a preliminary study of the effect of humidity on powdery dryrot development was undertaken in the spring of 1915.

In the month of April, owing to the fact that heavy rains had been falling, the storage cellar of the Jerome Experiment Station was in a comparatively damp condition, the doors having been open for a considerable portion of the time to allow workmen to enter. At the same time the cellar under the Station laboratory building was being kept in a comparatively dry condition, while the air of the laboratory itself was very dry, owing to the fact that fire was being constantly maintained in the stove. One hundred and fifty Netted Gem tubers inoculated with *F. trichothecioides* were allowed to remain for several days in moist chambers until the fungus had well established itself, after which fifty tubers were removed to the potato storage cellar; fifty of the tubers were put in the cellar of the laboratory building, while the remaining fifty tubers were exposed to the dry air of the laboratory room. After six weeks the potatoes were examined. Though the temperature had been very favorable for the development of the rot, the fifty tubers left in the dry laboratory room showed no apparent advance in the decay from the time they had been removed from the moist chambers. Those in the laboratory cellar showed but a very slight advance in the decay, while those in the storage cellar showed well-defined pockets of dryrot, each tuber being from one-eighth to one-fourth decayed. This preliminary experiment shows that the drier the atmosphere the less will be the decay in storage from this cause.

## DISINFECTION OF POTATO STOCK BEFORE STORING

In order to learn whether the progress of powdery dryrot in storage could be inhibited by disinfecting the potatoes before storage, the following experiments were set up. Both bruised and sound tubers were employed. The bruised ones had been injured in the field during the process of digging. Fumigation with formaldehyde gas was the method of disinfection used. The potatoes were fumigated in an air-tight room at a temperature of about 60° F. To produce the formaldehyde fumes the following formula was employed: Formaldehyde (40 per cent), 3 pints; potassium permanganate, 23 ounces for each 1,000 cubic feet of space. The potatoes were arranged in two lots as follows:

Lot 1 in trays, each tray holding about 50 pounds of potatoes. One tray each of bruised tubers of Early Rose, Improved Peachblow, Peoples, Netted Gem, and Pearl, and three trays of bruised tubers of Idaho Rural; also a similar number of trays of sound tubers of each variety.

Lot 2 in sacks, each holding about 100 pounds. One sack each of bruised tubers of Early Rose, Improved Peachblow, Peoples, Netted Gem, and Pearl and two sacks of bruised tubers of Idaho Rural; also a similar number of sound tubers of each variety. The potatoes were fumigated for 24 hours and then placed in storage. As a check, a similar number of trays and sacks of bruised tubers of each variety and a similar number of trays and sacks of sound tubers of each variety were put in storage without fumigation. The period of storage was from November 1, 1913, to May 10, 1914. The cellar was well ventilated and comparatively dry. The temperature throughout the storage period ranged from 0° as a minimum to 7.8° C. as a maximum.

A careful examination of the potatoes at the end of the storage period revealed the fact that a slight amount of decay had taken place in all of the bruised tubers, whether fumigated or unfumigated. Cultures were made from a large number of infected tubers, and the fungus *F. trichothecioides* was obtained. No apparent difference was noted between the fumigated and the unfumigated lots, and there was no decay in any of the unbruised tubers, whether fumigated or not. The unfumigated potatoes, however, sound or bruised, presented a much better appearance than the fumigated potatoes, owing to the injuries in the form of sunken spots which appeared on most of the fumigated tubers caused by the action of the formaldehyde fumes.

In the fall of 1915, other experiments to control powdery dryrot by disinfecting prior to storage were undertaken. On September 27, Idaho Rural tubers were dug for the experiment. One-half of the tubers were bruised in the field with the digging fork. Bruised and sound tubers were sacked separately. Twenty-five sacks of bruised tubers and twenty-five sacks of sound tubers were employed in the experiments. Each sack contained about 40 pounds of potatoes. The methods of disinfection were as follows: (1) The formaldehyde dip (1 pint of 40 per cent formaldehyde to 30 gallons of water). (2) The mercuric-chlorid dip (4 ounces of mercuric chlorid to 30 gallons of water). (3) Dusting with flowers of sulphur. (4) Dusting with air-slaked lime. The formaldehyde and mercuric-chlorid solutions were made up fresh for each disinfection.

The potatoes dug were divided into five lots, each lot consisting of five sacks of bruised tubers and five sacks of sound tubers. On September 27, a few minutes after digging, one sack of bruised tubers and one sack of sound tubers were placed in storage without disinfection. One sack each of bruised and sound tubers was dipped for two hours in the formaldehyde solution and then dried and put in storage. One sack each of bruised and sound tubers was dipped for two hours in the mercuric-chlorid solution, dried, and put in storage, one sack each of bruised and sound tubers was dusted with flowers of sulphur and put

in storage, and one sack each of bruised and sound tubers was dusted with lime and put in storage. On September 28, twenty-four hours after digging, the second lot, consisting of five sacks each of bruised and sound tubers, was treated in the same manner as that of the previous day and placed in storage. On September 29, forty-eight hours after digging, the third lot, consisting of five sacks each of bruised and sound tubers, was treated in the same manner as the first and second lots and placed in storage. On September 30, seventy-two hours after digging, the fourth lot, consisting of five sacks each of bruised and sound tubers, was treated in the same manner as lots 1, 2, and 3 on previous days and placed in storage. On October 1, ninety-six hours after digging, the fifth lot, consisting of five sacks each of bruised and sound tubers, was treated in the same manner as those on the previous days and was put in storage. In each case the disinfected potatoes were stored in the sacks in which they were disinfected. In order to give the experiment a severe test all five lots were stored for about six weeks in the anteroom of the storage cellar, where temperature and moisture conditions were favorable for dryrot development, after which they were transferred to the storage cellar proper.

On April 1, 2, and 3, 1916, examination of the potatoes was made. Each tuber was carefully examined to determine the presence or absence of decay. Wherever decay occurred, typical specimens were taken to the laboratory, and the presence of *F. trichothecioides* was determined by means of artificial cultures. All of the unbruised tubers, whether disinfected or not, were still wholly sound. The bruised tubers which were not disinfected presented essentially the same appearance in all lots. Those with deep bruises were usually from one-third to totally decayed. Those with shallow bruises in some cases showed no decay, but the majority showed at least slight decay. By "deep bruises" are meant those which penetrated the tuber tissue far enough to be partly closed up and covered over when the digging instrument was withdrawn; by "shallow bruises," those which were only skin deep, or which presented a comparatively clean cut surface. The condition of the disinfected, bruised tubers at the end of the storage period is shown in Table IV.

TABLE IV.—*Condition of the disinfected, bruised potato tubers at the end of the storage period*

| Time of disinfection. | Condition after disinfection with— | | | |
|---|---|---|---|---|
| | Mercuric chlorid. | Formaldehyde. | Lime. | Sulphur. |
| Immediately after digging. | No decay..... | No decay..... | Decay in most tubers with deep bruises. Shallow bruises healed over. | Presented the same appearance as those dusted with lime. |
| 24 hours after digging. | .....do..... | .....do..... | Presented the same appearance as those disinfected immediately after digging. | Do. |
| 48 hours after digging. | Very slight decay in most tubers with deep bruises. Shallow bruises healed over. | All tubers with deep bruises from one-third to one-half decayed. Tubers with shallow bruises showing slight or no decay. | .....do..... | Do. |
| 72 hours after digging. | Well-established dryrot in all tubers with deep bruises. Shallow bruises healed over. | Presented the same appearance as those disinfected 48 hours after digging. | .....do..... | Do. |
| 96 hours after digging. | All tubers with deep bruises from one-quarter to one-third decayed. Slight decay proceeding from shallow bruises. | A few tubers totally decayed. Balance presented the same appearance as those disinfected 48 hours after digging. | .....do..... | Do. |

It is evident from the results obtained by disinfecting potato stock prior to storage that it is possible to check the disease effectively, provided the disinfecting is done within 24 hours after digging. The solution of mercuric chlorid, which was the most effective, was fairly efficient 48 hours after digging. The formaldehyde solution gave the next best results and was thoroughly effective 24 hours after digging. It was of little or no value when applied from 48 to 96 hours after digging. There was little difference to be observed between the lots dusted with lime and those dusted with sulphur. Wherever the tuber bruises were of such a character that the lime or sulphur could reach and cover the bruised

surface, no decay occurred. The lime and sulphur dust did not always reach the deeper bruises and therefore was not effective in such cases. Disinfecting potatoes with mercuric chlorid or formaldehyde prior to storage should be of value when it is necessary to store seed potatoes in a poorly ventilated or improperly cooled storage cellar. Lime and sulphur are not recommended.

## SUMMARY

(1) Powdery dryrot, caused by *Fusarium trichothecioides*, is the most important storage-rot affecting potatoes in the irrigated West.

(2) *F. trichothecioides* under ordinary western field conditions does not attack any part of the growing potato plant. Potatoes in storage are attacked only through bruises.

(3) Planting badly infected seed potatoes greatly reduces the stand. A slight amount of infection in the seed piece does not cause any serious loss.

(4) The causal organism is at the present time apparently well distributed throughout western desert soils.

(5) *F. trichothecioides* does not develop at a temperature below 2° C. No loss from powdery dryrot occurs when the storage house is kept at this temperature, or lower. In a dry, well-ventilated storage house losses will be very slight at temperatures from 2° to 4° C. (35° to 40° F.).

(6) Where it is necessary to store seed potatoes in a poorly ventilated or improperly cooled storage house, the disease may be effectively checked by disinfecting the stock, prior to storage, with a solution of mercuric chlorid or formaldehyde, provided the disinfecting is done immediately, or within 24 hours after digging.

## PLATE CVIII

Fig. 1.—A potato tuber infected with powdery dryrot, showing the wrinkled condition of skin due to the decay of underlying tissues.

Fig. 2.—A potato tuber infected with powdery dryrot: Advanced stage. Note the presence of *Fusarium trichothecioides* on the surface of the decayed portion of the tuber.

Fig. 3.—Section through a potato tuber infected with powdery dryrot, showing the internal cavities filled with the mycelium and the spores of the fungus.

1
2
3

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# USE OF THE MOISTURE EQUIVALENT FOR THE INDIRECT DETERMINATION OF THE HYGROSCOPIC COEFFICIENT

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## INTRODUCTION

The maximum amount of soil water available for growth and for the maintenance of life in the case of ordinary crop plants appears to be approximately equal to the free water—the difference between the total amount of water and the hygroscopic coefficient—in those portions of the soil and the subsoil occupied by the roots (1, p. 121).[1] The hygroscopic coefficient (8, p. x; 10, p. 243) expresses the percentage of moisture contained in a soil which, in an air-dry condition, has been brought into a saturated atmosphere, kept at a constant temperature, and allowed to remain until in approximate equilibrium with this atmosphere.

Hilgard's method for the direct determination (10, p. 243; 11, p. 17) of the hygroscopic coefficient requires provision for the maintenance of a constant temperature in the room in which the absorption boxes are placed and also presents difficulties in insuring the actual saturation of the atmosphere in these boxes. Accordingly, any indirect method which gives results in satisfactory accord with those obtained by direct determination and at the same time requires only apparatus which is less inconvenient, either of installation or of operation, will prove useful.

Briggs and Shantz (7, p. 73) have recently proposed several indirect methods, and to the consideration of the reliability of one of these the present paper is devoted. These authors derived formulas for the indirect determination of what they designate the "wilting coefficient," defined as the moisture remaining in the soil in immediate contact with the roots when the permanent wilting of a plant occurs, from the moisture equivalent (6, p. 140; 4, p. 276), from the maximum water capacity as

[1] Reference is made by number to "Literature cited," p. 845.

defined by Hilgard (10, p. 256), and from the mechanical analysis. Subsidiary formulas for the indirect determination of the hygroscopic coefficient, following as a result of the interrelationships thus established, they report (7, p. 73) as follows:

$$\text{Hygroscopic coefficient} = \begin{cases} \text{Wilting coefficient} \times 0.68. \\ \text{Moisture equivalent} \times 0.37. \\ (\text{Maximum water capacity} - 21) \times 0.234. \\ (0.007\ \text{sand} + 0.082\ \text{silt} + 0.39\ \text{clay}). \end{cases}$$

As the mechanical analysis of a soil is a far more difficult and time-consuming operation than the determination of the hygroscopic coefficient, the latter could advantageously be calculated from the former only where this is already available, as, for example, in the reports of soil surveys. Even then there is a probability of introducting serious errors. Thus, with a series of loess soils (3, p. 411) it has recently been shown that the values for the hygroscopic coefficient calculated by the Briggs-Shantz formula agree satisfactorily with those obtained by direct determination only in the case of those samples which carry the smallest proportion of very fine sand. However, by altering the values assigned the sands there was obtained the following modified formula, which was found applicable to all the loess soils investigated.

Hygroscopic coefficient = 0.005 coarser fractions + 0.07 very fine sand + 0.82 silt + 0.39 clay.

"Coarser fractions" is here used to designate all soil particles having a diameter greater than 0.10 mm.

The wilting coefficient also is so inconvenient of determination that, unless it has to be determined for some other purpose, it will not be used to calculate the hygroscopic coefficient.

In connection with field studies of available soil moisture on the Nebraska loess, of which only a few data (1, p. 118) have as yet been published, one of us had arrived at conclusions so widely at variance with those of Briggs and Shantz, who, in somewhat similar studies, had employed the wilting coefficient, either determined directly or calculated from the moisture equivalent, that we suspected the explanation might lie in the differences in the values of the hygroscopic coefficient obtained for similar soils by our respective methods. However, as no moisture-equivalent apparatus was at that time available for our use we were unable then to decide the question. Now, using 135 samples of which hygroscopic coefficient determinations had been made at the Nebraska Experiment Station, we have determined the moisture equivalents, thus obtaining a definite answer to the question.

Lipman and Waynick (12) have recently reported both the moisture equivalents and the hygroscopic coefficients of 27 soils, and from these the ratios may be calculated. In so far as we are aware, there are no published data except those in the two articles mentioned from which the

relationship of the moisture equivalent to the hygroscopic coefficient can be computed.

## COMPUTATIONS FROM DATA OF BRIGGS AND SHANTZ

Briggs and Shantz have reported (7, p. 57–65) both the hygroscopic coefficients and the moisture equivalents in the case of 17 soils ranging in texture from a coarse sand with a hygroscopic coefficient of 0.5 to a clay loam with a value of 13.2. Their data, however, were not presented in such form as to show the concordance of the hygroscopic coefficients calculated from the moisture equivalents with those directly determined, and for this reason we consider it desirable to so present them (Table I).

TABLE I.—*Relation of the moisture equivalent to the hygroscopic coefficient shown by data of Briggs and Shantz*[1]

| Soil. | Type of soil. | Moisture equivalent. | Hygroscopic coefficient. | Ratio of moisture equivalent to hygroscopic coefficient. | Hygroscopic coefficient calculated from moisture equivalent. | Departure of calculated from determined hygroscopic coefficient. |
|---|---|---|---|---|---|---|
| 7 | Coarse sand..... | 1.6 | 0.5 | 3.20 | 0.6 | 0.1 |
| 2 | Fine sand..... | 4.7 | 1.5 | 3.13 | 1.7 | .2 |
| 8 | .....do..... | 5.5 | 2.3 | 2.39 | 2.0 | — .3 |
| 9 | .....do..... | 6.7 | 2.3 | 2.91 | 2.5 | .2 |
| 3 | Sandy loam..... | 9.7 | 3.5 | 2.77 | 3.6 | .1 |
| 10 | .....do..... | 11.9 | 4.4 | 2.70 | 4.4 | .0 |
| 4 | Fine sandy loam.. | 18.1 | 6.5 | 2.78 | 6.7 | .2 |
| 12 | Loam..... | 18.9 | 7.8 | 2.42 | 7.0 | — .8 |
| A | Sandy loam..... | 19.6 | 6.3 | 3.11 | 7.2 | .9 |
| B | Fine sandy loam.. | 19.9 | 6.6 | 3.01 | 7.3 | .7 |
| C | .....do..... | 22.1 | 7.5 | 2 94 | 8.2 | .7 |
| 5 | Loam..... | 25.0 | 9.8 | 2.55 | 9.2 | — .6 |
| D | .....do..... | 27.0 | 9.6 | 2.81 | 10.0 | .4 |
| 13 | Clay loam..... | 27.4 | 11.8 | 2.32 | 10.1 | —1.7 |
| 14 | .....do..... | 29.3 | 13.2 | 2.22 | 10.8 | —2.4 |
| E | .....do..... | 30.0 | 11.2 | 2.68 | 11.1 | — .1 |
| 6 | .....do..... | 30.2 | 11.4 | 2.65 | 11.2 | — .2 |
| | Mean [2]..... | ..... | ..... | 2.71 | ..... | ..... |
| | Maximum .. | ..... | ..... | 3.11 | ..... | ..... |
| | Minimum... | ..... | ..... | 2.22 | ..... | ..... |

[1] Derived from Briggs and Shantz (7, p. 57, 60, 65, Tables XVII, XIX, and XX).
[2] Omitting 7 and 2.

Excepting the two sands, 1 and 2, the ratio varies from 3.11 to 2.22, a range of 40 per cent, reaching a maximum in the case of a sandy loam with a hygroscopic coefficient of 6.3 and a minimum in a clay loam with the coefficient 13.2. In the case of the latter the value calculated from the mean ratio, 2.71, differs by 2.4 from that obtained by direct determination. Two of the four clay-loam samples give concordant and two rather discordant results, the divergence in the case of the latter being similar to that obtained from the mechanical analysis of many of the loess soils (3, p. 411).

## COMPUTATIONS FROM DATA OF LIPMAN AND WAYNICK

Lipman and Waynick report (12, p. 8–9) both the hygroscopic coefficients and the moisture equivalents on 27 samples used in the well-known so-called Tri-State Soil Exchange Experiment. The ratios, which evidently they did not compare, we show in Table II. These data have an added interest in that they are from the laboratory of the late Dr. Hilgard, who introduced the determination of the hygroscopic coefficient (8, 9, 10).

TABLE II.—*Relation of the moisture equivalent to the hygroscopic coefficient shown by the data of Lipman and Waynick*

HYGROSCOPIC COEFFICIENT [1]

| Depth. | California soil. | | | Kansas soil. | | | Maryland soil. | | |
|---|---|---|---|---|---|---|---|---|---|
| | In California. | In Kansas. | In Maryland. | In California. | In Kansas. | In Maryland. | In California. | In Kansas. | In Maryland. |
| *Feet.* | | | | | | | | | |
| 1 | 8.55 | 8.29 | 6.68 | 12.12 | 10.74 | 11.00 | 5.97 | 5.15 | 4.69 |
| 2 | 8.67 | 7.69 | 8.44 | 12.42 | 12.38 | 11.68 | 6.82 | 5.82 | 7.66 |
| 3 | 8.98 | 8.68 | 9.04 | 11.28 | 10.54 | 11.18 | 8.87 | 6.75 | 9.23 |
| Average | 8.73 | 8.22 | 8.05 | 11.94 | 11.22 | 11.29 | 7.22 | 5.91 | 7.19 |

MOISTURE EQUIVALENT [2]

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 24.09 | 22.32 | 22.67 | 32.61 | 29.63 | 29.80 | 23.62 | 23.67 | 21.92 |
| 2 | 22.81 | 22.20 | 20.32 | 33.33 | 30.78 | 31.14 | 24.26 | 26.02 | 19.37 |
| 3 | 24.02 | 24.24 | 23.53 | 30.21 | 27.57 | 29.40 | 29.17 | 29.16 | 27.38 |
| Average | 23.64 | 22.92 | 22.17 | 32.05 | 29.33 | 30.11 | 25.68 | 26.28 | 22.89 |

RATIO OF MOISTURE EQUIVALENT TO HYGROSCOPIC COEFFICIENT

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2.82 | 2.69 | 3.39 | 2.69 | 2.76 | 2.71 | 3.96 | 4.60 | 4.67 |
| 2 | 2.63 | 2.89 | 2.41 | 2.68 | 2.49 | 2.67 | 3.56 | 4.47 | 2.53 |
| 3 | 2.67 | 2.79 | 2.60 | 2.68 | 2.61 | 2.63 | 3.29 | 4.32 | 2.97 |
| Average | 2.71 | 2.79 | 2.80 | 2.68 | 2.62 | 2.67 | 3.60 | 4.46 | 3.39 |

[1] From Lipman and Waynick (12, p. 8, Table I). [2] From Lipman and Waynick (12, p. 9, Table II).

The average ratio for the 27 samples is 3.08, with a minimum of 2.41 and a maximum of 4.67, a range of 93 per cent. On inspection of Tables I and II it will be seen that for the Kansas soils the ratio varies only between 2.49 and 2.76, and for the California soils between 2.41 and 3.39, with an average for these 18 samples of 2.72, which is practically identical with the mean found by Briggs and Shantz—viz, 2.71.

In the case of the 9 samples of Maryland soils, the ratio varies from 2.53 to 4.67, with an average of 3.75. As none of the samples is to be considered lighter in texture than a loam or heavier than a clay loam, any ratio sufficiently accurate for ordinary purposes should apply to all of them.

## EXPERIMENTAL WORK

The moisture equivalents were determined according to Briggs and Shantz (7, p. 57), bringing the soils into equilibrium with a force 1,000 times that of gravity, using a centrifuge (6, p. 141) made according to specifications kindly furnished by Dr. L. J. Briggs, of the Bureau of Plant Industry. The determination of the moisture equivalent has been found to be convenient of execution, and the results from day to day are very concordant.

In Table III are given the moisture equivalent, the hygroscopic coefficient, the ratio of these to one another, and the content of organic matter in 36 samples. The soils were collected from 30 virgin prairie fields in Nebraska, 5 near each of the six towns indicated in the table. All are from fields classified by the United States Bureau of Soils either as Marshall silt loams or as Colby silt loam. In each field 10 borings were made to a depth of 6 feet and composite samples prepared of each foot section, thus securing 6 samples from each field. From these were prepared the samples used in this work, equal weights of the corresponding 5 field samples being combined. The details of the method of sampling are reported elsewhere (2, p. 204). In the same article (2, p. 215) are given the hygroscopic coefficients for the foot sections from each of all the fields. Each value in B of Table III represents the average of 10 determinations. The data on the organic matter reported in D of the table were calculated from the organic carbon reported in the same article (2, p. 228; organic matter = C × 1.724). The data on the moisture equivalents are the means of duplicate determinations.

The ratio (Table III–C) averages 2.38, varying from 2.14 to 2.73, a quite similar, although somewhat narrower, range than that found by Briggs and Shantz. In general, in each area it is highest in the surface foot as though influenced by the proportion of the organic matter.

TABLE III.—*Moisture equivalent, hygroscopic coefficient, ratio of these two values, and organic-matter content of the foot sections from six different areas in Nebraska*

(A) MOISTURE EQUIVALENT

| Depth. | Wauneta. | McCook. | Holdrege. | Hastings. | Lincoln. | Weeping Water. | Average. |
|---|---|---|---|---|---|---|---|
| *Feet.* | | | | | | | |
| 1 | 22.3 | 24.0 | 26.7 | 26.2 | 30.7 | 30.3 | 26.7 |
| 2 | 22.1 | 24.8 | 27.6 | 28.6 | 31.5 | 31.2 | 27.6 |
| 3 | 23.0 | 24.6 | 26.8 | 28.2 | 29.2 | 30.9 | 27.1 |
| 4 | 23.3 | 23.6 | 25.1 | 26.9 | 27.8 | 29.2 | 26.0 |
| 5 | 21.1 | 22.5 | 24.1 | 26.5 | 28.3 | 28.2 | 25.1 |
| 6 | 19.8 | 22.1 | 24.0 | 26.6 | 26.3 | 28.3 | 24.8 |
| Average | 21.9 | 23.6 | 25.7 | 27.2 | 29.3 | 29.7 | 26.2 |

(B) HYGROSCOPIC COEFFICIENT

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 9.1 | 10.0 | 10.1 | 9.6 | 12.0 | 12.1 | 10.5 |
| 2 | 9.6 | 10.9 | 11.2 | 11.6 | 14.4 | 13.7 | 11.9 |
| 3 | 9.7 | 10.7 | 11.3 | 12.4 | 13.6 | 13.9 | 11.9 |
| 4 | 9.9 | 9.7 | 10.2 | 11.1 | 13.0 | 13.0 | 11.1 |
| 5 | 9.0 | 9.1 | 9.6 | 10.7 | 12.8 | 12.6 | 10.6 |
| 6 | 8.3 | 9.1 | 9.4 | 10.7 | 12.7 | 12.5 | 10.5 |
| Average | 9.3 | 9.9 | 10.3 | 11.0 | 13.1 | 13.0 | 11.1 |

(C) RATIO OF MOISTURE EQUIVALENT TO HYGROSCOPIC COEFFICIENT

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 2.45 | 2.40 | 2.64 | 2.73 | 2.56 | 2.50 | 2.55 |
| 2 | 2.30 | 2.28 | 2.46 | 2.47 | 2.19 | 2.28 | 2.32 |
| 3 | 2.37 | 2.30 | 2.37 | 2.27 | 2.15 | 2.22 | 2.28 |
| 4 | 2.35 | 2.43 | 2.46 | 2.42 | 2.14 | 2.25 | 2.34 |
| 5 | 2.34 | 2.47 | 2.51 | 2.48 | 2.21 | 2.24 | 2.38 |
| 6 | 2.39 | 2.43 | 2.55 | 2.49 | 2.23 | 2.26 | 2.39 |
| Average | 2.37 | 2.38 | 2.50 | 2.48 | 2.25 | 2.29 | 2.38 |

(D) PERCENTAGE OF ORGANIC MATTER

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 2.77 | 2.85 | 3.90 | 3.55 | 4.96 | 4.98 | 3.83 |
| 2 | 1.38 | 1.44 | 1.86 | 1.81 | 2.28 | 3.02 | 1.96 |
| 3 | 1.09 | .97 | 1.01 | .98 | 1.14 | 1.38 | 1.09 |
| 4 | .79 | .59 | .66 | .60 | .60 | .83 | .68 |
| 5 | .55 | .48 | .41 | .41 | .43 | .45 | .45 |
| 6 | .45 | .36 | .36 | .31 | .40 | .36 | .37 |
| Average | 1.17 | 1.11 | 1.37 | 1.28 | 1.63 | 1.84 | 1.40 |

Table IV shows the values for the hygroscopic coefficients calculated from the moisture equivalents, using the Briggs-Shantz formula, and the departure from those directly determined. In all cases the values are more or less too low; using these there might appear to be as much as from 1.0 to 2.8 per cent of free water in the case of a subsoil which actually carried none.

TABLE IV.—*The hygroscopic coefficients calculated from the moisture equivalents and the departure of these from the values obtained by direct determination*

(A) CALCULATED HYGROSCOPIC COEFFICIENTS

| Depth. | Wauneta. | McCook. | Holdrege. | Hastings. | Lincoln. | Weeping Water. | Average. |
|---|---|---|---|---|---|---|---|
| *Feet.* | | | | | | | |
| 1 | 8.2 | 8.9 | 9.9 | 9.7 | 11.3 | 11.2 | 9.9 |
| 2 | 8.2 | 9.2 | 10.2 | 10.6 | 11.6 | 11.5 | 10.2 |
| 3 | 8.5 | 9.1 | 9.9 | 10.4 | 10.8 | 11.4 | 10.0 |
| 4 | 8.6 | 8.7 | 9.3 | 9.9 | 10.3 | 10.8 | 9.6 |
| 5 | 7.8 | 8.3 | 8.9 | 9.8 | 10.4 | 10.4 | 9.3 |
| 6 | 7.3 | 8.2 | 8.9 | 9.8 | 10.4 | 10.4 | 9.2 |
| Average | 8.1 | 8.7 | 9.5 | 10.0 | 10.8 | 11.0 | 9.7 |

(B) DEPARTURE FROM DIRECTLY DETERMINED VALUES

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | −0.9 | −1.1 | −0.2 | −0.1 | −0.7 | −0.9 | −0.6 |
| 2 | −1.4 | −1.7 | −1.0 | −1.0 | −2.8 | −2.2 | −1.7 |
| 3 | −1.2 | −1.6 | −1.4 | −2.0 | −2.8 | −2.5 | −1.9 |
| 4 | −1.3 | −1.0 | − .9 | −1.2 | −2.7 | −2.2 | −1.5 |
| 5 | −1.2 | − .8 | − .7 | − .9 | −2.4 | −2.2 | −1.3 |
| 6 | −1.0 | − .9 | − .5 | − .9 | −2.3 | −2.1 | −1.3 |
| Average | −1.2 | −1.2 | − .8 | −1.0 | −2.3 | −2.0 | −1.4 |

Table V gives similar data on another set of samples from the same 30 fields. These consisted of 1-inch sections from the surface foot (2, p. 206). In the case of these, however, each datum on hygroscopic coefficients as well as on moisture equivalents is the mean of only duplicate determinations. The ratio averages 2.75, compared with 2.71 found by Briggs and Shantz (Table I), and varies from 2.33 to 3.29, a range of 41 per cent, compared with 40 found by them with their 17 soils. Their samples also were probably surface soils rather than subsoils, such as predominate in Table III. In the inch sections, as in the foot sections, a decrease in the ratio is to be observed in passing from the surface to the subsoil. This may be attributed to the organic matter which appears to have a marked influence upon the moisture equivalent, although it shows little effect upon the hygroscopic coefficient (2, p. 217). Briggs and McLane (5, p. 18), found that organic matter had practically the same effect upon the moisture equivalent as an equal amount of clay.

TABLE V.—*Moisture equivalent, hygroscopic coefficient, ratio of these two values, and the organic content of the inch sections of the surface foot*

(A) MOISTURE EQUIVALENT

| Depth. | Wauneta. | McCook. | Holdrege. | Hastings. | Lincoln. | Weeping Water. | Average. |
|---|---|---|---|---|---|---|---|
| *Inches.* | | | | | | | |
| 1 | 24.4 | 24.3 | 31.6 | 31.5 | 31.0 | 32.7 | 29.2 |
| 2 | 22.3 | 22.2 | 29.6 | 27.6 | 30.0 | 31.5 | 27.2 |
| 3 | 22.5 | 22.9 | 28.3 | 27.3 | 30.2 | 31.3 | 27.1 |
| 4 | 22.3 | 23.3 | 28.3 | 27.6 | 30.5 | 30.6 | 27.1 |
| 5 | 22.6 | 24.5 | 27.5 | 27.3 | 29.9 | 31.7 | 27.2 |
| 6 | 22.5 | 25.7 | 27.7 | 27.8 | 30.0 | 31.9 | 27.6 |
| 7 | 22.3 | 25.4 | 28.1 | 27.5 | 30.1 | 32.4 | 27.6 |
| 8 | 22.3 | 25.6 | 27.9 | 27.3 | 30.3 | 31.9 | 27.5 |
| 9 | 22.8 | 27.2 | 28.0 | 27.6 | 30.9 | 31.4 | 28.0 |
| 10 | 22.8 | 27.3 | 28.2 | 27.4 | 30.7 | 32.2 | 28.1 |
| 11 | 23.0 | 27.9 | 28.0 | 27.9 | 30.0 | 31.9 | 28.1 |
| 12 | 23.0 | 26.8 | 28.1 | 28.4 | 30.8 | 32.3 | 28.2 |
| Average | 22.7 | 25.2 | 28.4 | 27.9 | 30.4 | 31.8 | 27.7 |

(B) HYGROSCOPIC COEFFICIENT

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 8.5 | 8.5 | 10.9 | 10.9 | 11.5 | 11.5 | 10.3 |
| 2 | 8.2 | 8.3 | 10.3 | 9.7 | 11.2 | 11.0 | 9.8 |
| 3 | 8.2 | 8.4 | 9.9 | 8.9 | 11.0 | 11.0 | 9.6 |
| 4 | 8.3 | 8.3 | 9.5 | 8.5 | 11.1 | 11.1 | 9.5 |
| 5 | 8.2 | 8.7 | 9.4 | 8.3 | 11.4 | 11.2 | 9.5 |
| 6 | 8.6 | 9.3 | 9.4 | 9.0 | 11.8 | 11.2 | 9.9 |
| 7 | 8.7 | 9.5 | 9.7 | 9.5 | 11.9 | 11.5 | 10.1 |
| 8 | 8.8 | 9.8 | 9.9 | 9.5 | 12.1 | 12.1 | 10.4 |
| 9 | 8.6 | 9.9 | 10.0 | 9.5 | 13.0 | 12.3 | 10.6 |
| 10 | 8.8 | 10.3 | 10.4 | 9.7 | 12.6 | 12.6 | 10.7 |
| 11 | 9.0 | 10.3 | 10.2 | 10.0 | 12.9 | 12.5 | 10.8 |
| 12 | 8.7 | 10.2 | 10.2 | 10.2 | 13.1 | 12.8 | 10.9 |
| Average | 8.6 | 9.3 | 10.0 | 9.5 | 12.0 | 11.7 | 10.2 |

(C) RATIO OF MOISTURE EQUIVALENT TO HYGROSCOPIC COEFFICIENT

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 2.87 | 2.86 | 2.90 | 2.89 | 2.70 | 2.84 | 2.84 |
| 2 | 2.72 | 2.68 | 2.87 | 2.85 | 2.68 | 2.86 | 2.78 |
| 3 | 2.74 | 2.73 | 2.86 | 3.07 | 2.75 | 2.85 | 2.84 |
| 4 | 2.69 | 2.81 | 2.98 | 3.25 | 2.75 | 2.76 | 2.87 |
| 5 | 2.76 | 2.82 | 2.93 | 3.29 | 2.62 | 2.83 | 2.88 |
| 6 | 2.62 | 2.76 | 2.95 | 3.09 | 2.54 | 2.85 | 2.79 |
| 7 | 2.56 | 2.67 | 2.90 | 2.89 | 2.53 | 2.82 | 2.73 |
| 8 | 2.53 | 2.61 | 2.82 | 2.87 | 2.50 | 2.64 | 2.66 |
| 9 | 2.65 | 2.75 | 2.80 | 2.91 | 2.38 | 2.56 | 2.68 |
| 10 | 2.59 | 2.65 | 2.71 | 2.83 | 2.44 | 2.56 | 2.63 |
| 11 | 2.56 | 2.71 | 2.74 | 2.79 | 2.33 | 2.55 | 2.62 |
| 12 | 2.64 | 2.63 | 2.75 | 2.78 | 2.35 | 2.52 | 2.61 |
| Average | 2.66 | 2.72 | 2.85 | 2.96 | 2.55 | 2.72 | 2.74 |

TABLE V.—*Moisture equivalent, hygroscopic coefficient, ratio of these two values, and the organic content of the inch sections of the surface foot*—Continued.

(D) PERCENTAGE OF ORGANIC MATTER

| Depth. | Wauneta. | McCook. | Holdrege. | Hastings. | Lincoln. | Weeping Water. | Average. |
|---|---|---|---|---|---|---|---|
| *Inches.* | | | | | | | |
| 1 | 4.91 | 4.17 | 7.93 | 7.79 | 8.10 | 7.79 | 6.78 |
| 2 | 3.64 | 3.35 | 6.03 | 5.46 | 6.29 | 6.39 | 5.19 |
| 3 | 3.19 | 3.27 | 4.95 | 4.45 | 5.70 | 5.60 | 4.53 |
| 4 | 2.88 | 3.14 | 4.22 | 3.93 | 5.37 | 5.29 | 4.14 |
| 5 | 2.55 | 2.84 | 3.74 | 3.50 | 4.89 | 4.87 | 3.73 |
| 6 | 2.52 | 2.66 | 3.46 | 3.20 | 4.72 | 4.56 | 3.52 |
| 7 | 2.26 | 2.48 | 3.05 | 3.09 | 4.31 | 4.08 | 3.21 |
| 8 | 2.19 | 2.31 | 2.97 | 2.88 | 4.12 | 4.03 | 3.08 |
| 9 | 2.12 | 2.17 | 2.79 | 2.74 | 3.98 | 3.95 | 2.96 |
| 10 | 1.97 | 2.00 | 2.67 | 2.64 | 3.59 | 3.76 | 2.77 |
| 11 | 1.77 | 1.84 | 2.58 | 2.62 | 3.40 | 3.69 | 2.65 |
| 12 | 1.77 | 1.72 | 2.50 | 2.50 | 3.26 | 3.60 | 2.56 |
| Average | 2.67 | 2.69 | 3.90 | 3.72 | 4.77 | 4.81 | 3.76 |

The samples reported in Table VI are partly from the loess of Nebraska and partly from the residual soils of that State. A few are from New Mexico, Arizona, and California. The data upon both the hygroscopic coefficient and the moisture equivalent are the means of 5 to 10 concordant determinations. Nine of the samples are from the surface 6 to 12 inches, and the seven others from the subsoil. The range in texture represented by them is much the same as that of the soils reported by Briggs and Shantz (Table I).

TABLE VI.—*Relation of the moisture equivalent to the hygroscopic coefficient in a series of soils showing a wide range in texture*

| Soil No. | Description of soil. | Moisture equivalent. | Hygroscopic coefficient. | Ratio of moisture equivalent to hygroscopic coefficient. | Hygroscopic coefficient calculated from moisture equivalent.[1] | Departure of calculated from found hygroscopic coefficient. |
|---|---|---|---|---|---|---|
| 1 | Desert sand, Palm Springs, Cal... | 1.6 | 0.9 | 1.77 | 0.6 | −0.3 |
| 2 | Sandy subsoil, Palm Springs, Cal. | 2.8 | 1.1 | 2.54 | 1.0 | − .1 |
| 3 | Desert sand, Orogrande, N. Mex.. | 3.0 | 1.7 | 1.76 | 1.1 | − .6 |
| 4 | Sandy surface, W. Nebraska..... | 7.9 | 3.3 | 2.39 | 2.9 | − .4 |
| 5 | Sandy subsoil (A), W. Nebraska.. | 7.2 | 3.4 | 2.12 | 2.6 | − .8 |
| 6 | Sandy subsoil (B), W. Nebraska .. | 7.5 | 3.4 | 2.21 | 2.8 | − .6 |
| 7 | Sandy loam subsoil, W. Nebraska. | 13.5 | 5.6 | 2.41 | 5.0 | − .6 |
| 8 | Sandy loam surface, W. Nebraska. | 16.8 | 7.1 | 2.37 | 6.2 | − .9 |
| 9 | Silt loam subsoil (A),W. Nebraska. | 19.7 | 7.6 | 2.59 | 7.3 | − .3 |
| 10 | Silt loam subsoil (B),W. Nebraska. | 21.2 | 8.2 | 2.59 | 7.8 | − .4 |
| 11 | Red loam surface, Cuervo, N. Mex. | 19.2 | 10.0 | 1.92 | 7.1 | −2.9 |
| 12 | Silt loam surface (A),W. Nebraska. | 22.5 | 10.1 | 2.23 | 8.3 | −1.8 |
| 13 | Silt loam surface, E. Nebraska.... | 27.8 | 10.2 | 2.73 | 10.3 | .1 |
| 14 | Silt loam surface (B),W. Nebraska. | 24.1 | 10.5 | 2.30 | 8.9 | −1.6 |
| 15 | Adobe surface, McNeal, Ariz..... | 25.8 | 12.9 | 2.00 | 9.6 | −3.3 |
| 16 | Silt loam subsoil, E. Nebraska.... | 29.5 | 13.3 | 2.22 | 10.9 | −2.4 |

[1] Using Briggs and Shantz formula M. E.=hyg. coeff. ×2.71.

Excepting the two sands, 1 and 3, the average ratio of moisture equivalent to hygroscopic coefficient is 2.33, with a maximum of 2.73 and a minimum of 1.92. The lowest ratios are shown by the arid or semiarid soils, 1, 3, 11, and 15. The exceptional behavior of 11 and 15 is not to be attributed to error of determination, as, after finding these exceptional ratios, we made repeated determinations of both values. The ratios found for both subsoils and surface soils from Nebraska are quite similar to those reported in Table III, the average ratio, 2.38, being identical with that obtained for the 36 loess samples.

If the two sands, 1 and 3, in Table VI, be omitted, the variation of our ratios in Tables III, V, and VI are of much the same order as those of Briggs and Shantz, shown in Table I. Thus, the divergence in our conclusions as to the availability to plants of the portion of the soil moisture lying between the hygroscopic coefficient and the wilting coefficient is not to be explained by any differences in our respective methods of arriving at the value of the hygroscopic coefficient. Neither are there sufficient reasons to attribute it to the particular range of soils with which we have worked, for the data above show that our soils range as widely as those which they have employed.

Their data, as well as our own work, make it evident that in any accurate experiments to determine the relation of the nonavailable water of the soil to the hygroscopic coefficient it is not permissible to calculate the value of the latter from the moisture equivalent, unless a previous thorough investigation has been made to determine just what formula is applicable to the soil type in question. From the data of Lipman and Waynick it would appear that in the case of certain soils this indirect method would be scarcely allowable for even the crudest studies on soil moisture. However, in the case of any extensive study, involving many soil types, the same general conclusions as to the relation of the nonavailable moisture to the hygroscopic coefficient are to be expected, no matter whether the latter value be directly determined or be calculated from the moisture equivalent by the Briggs-Shantz or by some more satisfactory formula.

## COMPUTATION OF THE MOISTURE EQUIVALENT FROM THE MECHANICAL ANALYSIS

Table VII shows the concordance of the moisture equivalents directly determined with the values computed from the mechanical analyses in the cases of the loess samples reported in Table III, using the formula proposed by Briggs and Shantz:

Moisture equivalent = 0.02 sands + 0.22 silt + 1.05 clay, and also a modified form of this formula:

Moisture equivalent = 0.14 sands + 0.27 silt + 0.53 clay. In these formulas "sands" include particles ranging from 2 to 0.05 mm. in diameter. The separates referred to in the table as "coarser fractions" include the particles ranging from 2 to 0.10 mm. It will be seen that the

formula of Briggs and Shantz gives values too low for the coarsest textured members of the series and too high for the finest textured. In the modified formula the value assigned to the clay is lowered, that to the "sands" much increased, and that to the silt slightly raised. This formula gives results in close concordance with the directly determined values. The explanation of the need of altering the values is not far to seek. As has already been pointed out in connection with the computation of the hygroscopic coefficients from the mechanical analyses of the same samples (3, p. 406), the material coarser than silt is chiefly very fine sand, consisting mainly of particles but little larger than the upper limit for silt, while the so-called "clay" contains a very large proportion of silt particles with a diameter not much less than 0.005 mm.

Briggs and McLane (5, p. 21), in applying their generalized formula based upon the analysis of 104 soils, found that for the Marshall series it was necessary to give the clay a lower value and also to make allowance for the content of organic matter. As has been mentioned above, the samples in Table VII belong to the Marshall and Colby series.

Thus, it appears that if the mechanical analyses are to be used for the computation of moisture equivalents, it will be necessary, at least in the case of some widely differing soil types, to employ several different formulas.

TABLE VII.—*Concordance of the values for the moisture equivalent obtained by computation from the mechanical analysis with those directly determined*

WAUNETA

| Depth. | Coarser fract (2.0—0.1 mm.). | Very fine sand (0.1—0.05 mm.). | Silt (0.05—0.005 mm.). | Clay (0.005—0.000 mm.). | Moisture equivalent. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Determined. | Computed by formula. | | Departure, using formula. | |
| | | | | | | B. and S.[1] | Mod.[2] | B. and S. | Mod. |
| *Feet.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | | | | | |
| 1 | 4.8 | 48.7 | 41.2 | 5.4 | 22.3 | 15.9 | 21.5 | −5.4 | −0.8 |
| 2 | 2.4 | 47.8 | 43.3 | 6.6 | 22.1 | 17.5 | 22.2 | −4.6 | .1 |
| 3 | 2.0 | 46.8 | 43.8 | 7.5 | 23.0 | 18.5 | 22.6 | −4.5 | −.4 |
| 4 | 1.7 | 47.6 | 41.3 | 9.5 | 23.3 | 20.1 | 23.1 | −3.2 | −.2 |
| 5 | 1.5 | 50.0 | 43.6 | 4.9 | 21.1 | 15.8 | 21.6 | −5.3 | .5 |
| 6 | 1.3 | 54.9 | 39.8 | 4.2 | 19.8 | 14.3 | 20.8 | −5.5 | 1.0 |
| Average. | 2.3 | 49.3 | 42.2 | 6.3 | 21.9 | 17.0 | 21.9 | −4.9 | 0 |

M'COOK

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3.7 | 39.0 | 48.6 | 8.7 | 24.0 | 20.7 | 23.7 | −3.3 | −0.3 |
| 2 | 2.6 | 37.8 | 50.1 | 9.5 | 24.8 | 21.8 | 24.2 | −3.0 | −.6 |
| 3 | 1.3 | 36.4 | 53.9 | 8.4 | 24.6 | 21.4 | 24.3 | −3.2 | −.3 |
| 4 | 1.4 | 38.9 | 52.4 | 7.4 | 23.6 | 20.0 | 23.7 | −3.6 | .1 |
| 5 | 1.8 | 39.3 | 52.6 | 6.3 | 22.5 | 19.0 | 23.3 | −3.5 | .8 |
| 6 | 1.2 | 40.4 | 51.8 | 6.6 | 22.1 | 19.2 | 23.3 | −2.9 | 1.2 |
| Average. | 2.0 | 38.6 | 51.6 | 7.8 | 23.6 | 20.4 | 23.7 | −3.2 | .1 |

[1] Moisture equivalent=0.02 sands+0.22 silt+1.05 clay.

[2] Moisture equivalent=0.14 sands+0.27 silt+0.53 clay.

TABLE VII.—*Concordance of the values for the moisture equivalent obtained by computation from the mechanical analysis with those directly determined*—Continued.

HOLDREGE

| Depth. | Coarser fract (2.1—0.1 mm.). | Very fine sand (0.1—0.05 mm.). | Silt (0.05—0.005 mm.). | Clay (0.005—0.000 mm ). | Moisture equivalent. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Determined. | Computed by formula. | | Departure, using formula. | |
| | | | | | | B. and S. | Mod. | B. and S. | Mod. |
| *Feet.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | | | | | |
| 1 | 2.8 | 25.9 | 64.6 | 6.7 | 26.7 | 21.8 | 25.0 | −4.9 | −1.7 |
| 2 | 1.3 | 24.6 | 62.9 | 11.6 | 27.6 | 26.1 | 26.5 | −1.5 | −1.2 |
| 3 | .8 | 26.5 | 62.5 | 10.5 | 26.8 | 25.3 | 26.2 | −1.5 | − .6 |
| 4 | .9 | 27.8 | 64.8 | 6.4 | 25.1 | 21.6 | 24.9 | −3.5 | − .2 |
| 5 | 2.5 | 31.7 | 60.0 | 5.8 | 24.1 | 20.0 | 24.1 | −4.1 | .0 |
| 6 | 2.4 | 31.1 | 60.7 | 5.8 | 24.0 | 20.1 | 24.2 | −3.9 | .2 |
| Average. | 1.8 | 27.9 | 62.6 | 7.7 | 25.7 | 22.5 | 25.1 | −3.2 | − .6 |

HASTINGS

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3.9 | 23.9 | 64.6 | 7.6 | 26.2 | 22.8 | 25.4 | −3.4 | −0.8 |
| 2 | 2.7 | 20.3 | 64.5 | 12.5 | 28.6 | 27.8 | 27.3 | − .8 | −1.5 |
| 3 | 2.3 | 22.2 | 61.9 | 13.6 | 28.2 | 28.4 | 27.4 | .2 | − .8 |
| 4 | 2.1 | 21.5 | 62.4 | 14.0 | 26.9 | 28.9 | 27.6 | 2.0 | .7 |
| 5 | 2.4 | 20.9 | 66.7 | 10.0 | 26.5 | 25.6 | 26.6 | − .9 | .1 |
| 6 | 2.2 | 20.7 | 67.2 | 9.9 | 26.6 | 25.6 | 26.6 | −1.0 | 0 |
| Average. | 2.6 | 21.6 | 64.5 | 11.3 | 27.2 | 26.5 | 26.8 | −0.7 | −0.4 |

LINCOLN

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3.8 | 13.5 | 68.0 | 14.8 | 30.7 | 30.8 | 28.6 | 0.1 | −2.1 |
| 2 | 3.7 | 9.8 | 67.6 | 18.9 | 31.5 | 35.0 | 30.2 | 3.5 | −1.3 |
| 3 | 3.4 | 9.3 | 68.0 | 19.3 | 29.2 | 35.5 | 30.4 | 6.3 | 1.2 |
| 4 | 3.2 | 9.6 | 68.1 | 18.9 | 27.8 | 35.1 | 30.2 | 7.3 | 2.4 |
| 5 | 3.7 | 9.9 | 69.4 | 17.0 | 28.3 | 33.4 | 29.7 | 5.1 | 1.4 |
| 6 | 3.9 | 9.5 | 70.2 | 16.5 | 28.3 | 33.0 | 29.6 | 4.7 | 1.5 |
| Average. | 3.6 | 10.3 | 68.5 | 17.6 | 29.3 | 33.8 | 29.8 | 4.5 | .5 |

WEEPING WATER

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.2 | 9.7 | 72.2 | 13.9 | 30.3 | 30.8 | 28.8 | 0.5 | −1.5 |
| 2 | 2.8 | 8.2 | 69.5 | 19.6 | 31.2 | 36.1 | 30.7 | 4.9 | − .5 |
| 3 | 1.0 | 13.8 | 66.7 | 18.6 | 30.9 | 34.5 | 29.9 | 3.6 | −1.0 |
| 4 | .6 | 14.9 | 67.0 | 17.6 | 29.2 | 33.5 | 29.6 | 4.3 | .4 |
| 5 | .5 | 14.7 | 67.9 | 17.0 | 28.2 | 33.1 | 29.5 | 4.9 | 1.3 |
| 6 | .5 | 15.0 | 67.5 | 17.1 | 28.3 | 33.1 | 29.5 | 4.8 | 1.2 |
| Average. | 1.7 | 12.7 | 68.5 | 17.3 | 29.7 | 33.5 | 29.7 | 3.8 | 0 |
| Average of all.. | 2.3 | 26.7 | 59.7 | 11.3 | 26.2 | 25.6 | 26.2 | −0.6 | 0 |

SUMMARY

The hygroscopic coefficient may in most cases be calculated from the moisture equivalent with sufficient accuracy to permit its use in soil-moisture studies. For certain types of soil, however, the ratio departs so widely from that assigned by Briggs and Shantz that the indiscriminate use of the latter value does not seem permissible. Before employing this indirect method for the determination of the hygroscopic coefficient in connection with soil-moisture studies the ratio should be experimentally established for each of the particular types of soil involved.

The effect of considerable quantities of organic matter is, in general, to give the ratio of the moisture equivalent to the hygroscopic coefficient a higher value.

In the case of any extensive study of soil moisture involving many soil types the same general conclusions as to the relation of the non-available moisture to the hygroscopic coefficient are to be expected no matter whether the latter value be directly determined or be calculated from the moisture equivalent by the Briggs-Shantz formula.

For the calculation of the moisture equivalent from the mechanical analysis no general formula appears universally applicable, the formula needing modification according to the soil type to which it is to be applied.

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# THERSILOCHUS CONOTRACHELI, A PARASITE OF THE PLUM CURCULIO

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## INTRODUCTION

During the seasons of 1914 and 1915 the ichneumonid *Thersilochus conotracheli* Riley (Pl. CIX) has been by far the most abundant and effective parasite of the plum curculio at North East, Pa. In 1914 a very large percentage of the fruit on a few plum trees (*Prunus* spp.) that stand on the premises of the Bureau of Entomology laboratory at that place was infested by the curculio. In the spring the adults of the parasite were abundant on these trees and parasitized a large percentage of the curculio larvæ in fruit that was still on the trees when the parasites became active.

Under date of June 13, 1914, the writer's notes contain the following:

> Very few of the larvæ of this species have been found in host larvæ more than one-eighth inch long, although many of larger size than this have been examined. This indicates that the parasite does not begin oviposition until some time after the curculio has begun its attack on the fruit, and therefore does not exercise any control over the early curculio larvæ.

The season of 1915 found the curculio much reduced in numbers. The cold, wet season, however, retarded the emergence of the parasites to such an extent that they attacked only the latest of the larvæ, practically all of these being parasitized.

## HISTORICAL REVIEW

The first mention of *Thersilochus conotracheli* in literature appeared in 1871, when Riley (1) [1] published his original description of the species, referring it to the genus Porizon, and recorded it as a parasite of the plum curculio (*Conotrachelus nenuphar* Herbst) in New Jersey. Riley (2, p. 18) again referred to it in a paper written in German and published in St. Louis. In 1880 Gott (3, p. 57) reported that in his work with the plum curculio in Canada he had not found this parasite. Riley and Howard (4, p. 63–64) in 1889 referred the species to the genus Thersilochus, gave a brief life history, and recorded the species as nearly as

[1] Reference is made by number to "Literature cited," p. 855.

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abundant in some sections as *Sigalphus curculionis* Fitch. Thereafter until 1906 there were published apparently only two references to the species; one by Riley and Howard (5), which is a repetition of the original breeding record, and one by Harrington (6, p. 67), in which the insect is merely mentioned as a parasite of the plum curculio. In 1906 Johnson and Girault (7, p. 6) mentioned this insect in the account of their work on the plum curculio in New York, and accorded it small importance in the control of its host. Quaintance and Jenne (8, p. 147–148) in 1912 gave a résumé of previously published accounts, together with data on the abundance and emergence of adults in spring in New York and Pennsylvania. As showing the distribution of the species these authors list the following States: New York, New Jersey, Connecticut, Illinois, Missouri, and Kansas.

## HOSTS AND DISTRIBUTION

So far as is known the plum curculio is the only insect attacked by this species, published records showing it to have been reared from this host in Connecticut, New York, New Jersey, Pennsylvania, Illinois, Missouri, and Kansas. In addition to these States the writer has had material from Michigan.

## LIFE HISTORY OF THE SPECIES

### GENERATIONS

This species is single-brooded, the life cycle of one generation embracing the whole year. The adult stage is reached in the fall, but the perfect insect does not leave its cocoon until the following spring.

### EMERGENCE OF ADULTS

The adult *T. conotracheli* emerges from its cocoon from late May to the middle of June and very shortly begins the search for hosts.

### RELATIVE ABUNDANCE AND TIME OF EMERGENCE OF SEXES

The males begin to appear a few days ahead of the females, and the latter continue to emerge long after the last male. A lot of cocoons collected by the late A. G. Hammar at Douglas, Mich., in the spring of 1911 and reared by the writer at Vienna, Va., produced adults as indicated in Table I. This table shows the date and period of emergence, the comparative dates of emergence of males and females, and the proportion of the sexes.

TABLE 1.—*Emergence of adults of Thersilochus conotracheli from cocoons collected at Douglas, Mich., and reared at Vienna, Va., in 1911*

| No. | Date emerged. | Males. | Females. |
|---|---|---|---|
| 1 | May 22 | 2 | ..... |
| 2 | 23 | 1 | ..... |
| 4 | 25 | 14 | ..... |
| 6 | 27 | 11 | 1 |
| 7 | 28 | 1 | 2 |
| 8 | 29 | 1 | 2 |
| 9 | 30 | 2 | 2 |
| 10 | 31 | 2 | 8 |
| 11 | June 1 | 3 | 6 |
| 12 | 2 | 1 | 3 |
| 13 | 3 | ..... | 2 |
| 15 | 5 | ..... | 11 |
| 16 | 6 | ..... | 5 |
| 18 | 8 | ..... | 3 |
| 19 | 9 | ..... | 3 |
| 22 | 12 | ..... | 3 |
| 24 | 14 | ..... | 2 |
| 27 | 17 | ..... | 1 |
| 28 | 18 | ..... | 1 |
| 29 | 19 | ..... | 1 |
| 30 | 20 | ..... | 1 |
| 32 | 22 | ..... | 1 |
| 33 | 23 | ..... | 1 |
| 35 | 25 | ..... | 1 |
| 37 | 27 | ..... | 1 |
| Total | ..... | 38 | 61 |
| Average date of emergence | ..... | May 27 | June 7 |
| Percentage of sexes | ..... | 38.39 | 61.61 |

## LONGEVITY OF THE ADULT

Some data on the longevity of the adult were obtained in 1911 from specimens reared at Vienna, Va., from the cocoons collected by Mr. Hammar at Douglas, Mich. Only females were used. Ten of these were divided into three lots, as follows: Four were placed in vials without food or water, three with water, and three with dilute sugar sirup. Of those without food or water one lived 4 days, two lived 3 days, and one lived 1 day. One of those provided with water lived 7 days, one 6 days, and one 4 days. Those fed with sirup lived 10 days, 13 days, and 15 days. The confinement in the vials undoubtedly shortened the life, even of the sirup-fed specimens, but the figures show the necessity for both food and water; for those given clear water lived, on the average, more than twice as long as those without food or water, and those provided with sirup lived more than four and one-half times as long.

## STAGE OF HOST ATTACKED

Parasitization takes place while the curculio larva is still very small, probably from the time it hatches until in its burrowing into the fruit it gets beyond the reach of the ovipositor of the parasite.

### EFFECT OF PARASITISM ON HOST

Beyond possibly a temporary inactive condition induced by the sting, the oviposition by the parasite does not seem to injure the host. But shortly after the newly hatched larva begins to feed, the character of the body contents shows a considerable change. The adipose tissue loses its flocculent character and becomes a more homogeneous, more fluid mass, diluted by the blood. This same change in the host was noted by Timberlake (9) in his studies of *Limnerium validum* Cresson.

Parasitized larvæ at the time they leave the fruit are, as a rule, much smaller than healthy larvæ, although parasites have been found in hosts of nearly normal size. Whether this smaller size is due to the failure of the host to grow normally or to its failure to pass through all of its stages is a question which has not been determined.

### OVIPOSITION

In oviposition the female parasite, having found a curculio oviposition scar, raises her abdomen, at the same time releasing the ovipositor from its sheath and, directing it forward between her legs, thrusts it into the tunnel made by the curculio larva. If she can reach the larva, she pierces its skin and deposits within it a single egg. The act of oviposition is very brief, the longest observed having required about two minutes.

The female *T. conotracheli* has been observed repeatedly in the cages to attempt oviposition or rather to probe for possible hosts in abrasions of any sort in the skin of the plums provided. This apparently indicates that she can not recognize infallibly the typical scar made by the curculio in oviposition.

### INCUBATION PERIOD

No exact data on the incubation period are available, but that it is very short is indicated by the fact that in the many young larvæ examined in the course of the observations only one egg was found within a host, although they are very easily discovered. At the same time that the above-mentioned egg was discovered, another curculio larva, which had not traversed more than half an inch within the fruit, was found to contain a very young parasite larva. This also would indicate a short incubation period. A curculio larva exposed in a cage for one day, July 1–2, 1915, to the attack of *T. conotracheli* and dissected on July 7 was found to contain a very young larva of the parasite. This would indicate a maximum incubation period of six days, although it may have been even shorter than that.

## THE LARVA

POSITION IN RELATION TO HOST.—Throughout most of the larval life this species lives as an internal parasite, the larval parasite lying free within the body cavity of its host. When nearly full grown, however, it leaves the host and becomes temporarily an external feeder, draining from without the last trace of fluid from the body of its victim.

FEEDING PERIOD.—Because of the impossibility of following an individual parasite throughout its development, the determination of the exact duration of the various larval instars is very difficult and must be based on the average of many individuals. During this period of its life the larva molts four times. Larvæ of the first instar are to be found within curculio larvæ even as long as three days after the latter have finished feeding and entered the ground. In fact, it seems to be the rule that the first larval molt of the parasite takes place after the host has constructed its pupal cell. Apparently, however, this is not invariably true, since larvæ as old as the third instar have been removed from their hosts within three days of the time the latter entered the ground. Thereafter the development of the parasite is very rapid, for within 10 days it passes through its second, third, and fourth instars, and in some cases has left the body of its host and has begun the construction of its cocoon.

## DESTRUCTION OF SUPERNUMERARY LARVÆ.

Repeatedly in the dissection of the parasitized curculio larvæ more than one, sometimes several, first-instar larvæ of *T. conotracheli* have been found in a single host. Invariably, however, only one of these was in a healthy condition. The others were mostly dead and more or less inclosed in a mass of cells in the manner described by Timberlake (9, p. 75–76), and shown by him to be amebocytosis. In one case a still living but unhealthy larva partially inclosed by amebocytes was found in a curculio larva that also contained one healthy larva and two dead and completely invested larvæ. In no case, however, have all of the parasites been dead. Apparently the death of the parasite larvæ is not due to any protective adaptation on the part of the host, as suggested by Timberlake (9), for parasites in strange host species; for, as stated above, in no case were all larvæ killed, and in no case where but one egg was deposited within a host was the parasitism unsuccessful. The only source, therefore, of the destructive agency, whatever its nature, must lie within the surviving parasite larva. No explanation as to the nature of this agency is possible at this time.

### THE COCOON

The cocoon of *T. conotracheli* (fig. 1) is about 5 mm. long by about half as thick and oval in shape. It is constructed of tough reddish-brown silk.

FIG. 1.—*Thersilochus conotracheli:* Cocoon. Much enlarged.

### PUPATION AND TRANSFORMATION

About four or five days after the construction of the cocoon pupation takes place. When the transformation to the adult condition takes place was not determined by the present writer, but according to Quaintance and Jenne (8, p. 147–148) Mr. Fred Johnson found adults in cocoons at Youngstown, N. Y., in 1908, as early as August 24. As indicated above, the species hibernates in this condition within the cocoon.

## DESCRIPTION OF THE IMMATURE STAGES

### THE EGG

The egg (fig. 2) is oblong oval in shape, somewhat larger at the cephalic end, about 0.33 mm. in length by a little more than a fourth as wide, and slightly curved. A magnification of 215 diameters showed no sculpture of the chorion.

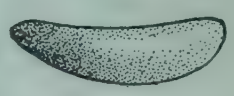

FIG. 2.—*Thersilochus conotracheli:* Egg. Highly magnified.

### THE LARVA

FIRST INSTAR.—The newly hatched larva (fig. 3) resembles in general appearance that of *Limnerium validum* as figured by Timberlake (9, p. 84).

The body consists of 13 segments, including the head and the long taillike caudal segment. The head is somewhat more than half as long as the rest of the body exclusive of the tail, which is somewhat longer than the head. The head is bent slightly downward from the general axis of the body. It is heavily chitinized and pale brown in color, considerably longer than wide, and strongly curved above so that the mouth opening is on the underside. The mouth parts consist of the heavily chitinized, acute, curved mandibles and the very delicate labrum, maxillæ, and labium. The exact form of the last three appendages is very difficult to ascertain with exactness, but they seem to be arranged about as in figure 4. The mandibles are very distinct even well within the head cavity, although their place of attachment is not clear. Ap-

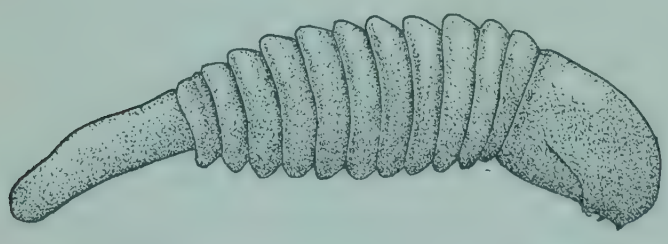

FIG. 3.—*Thersilochus conotracheli:* Larva of first instar. Highly magnified.

parently they are attached very close together at a point above the labium. During its first instar the larva undergoes a comparatively enormous increase in size, becoming ultimately 2 mm. in length and much distended, only the head and taillike appendage retaining their original dimensions. The approximate appearance of the parasite at this period of its development is shown in figure 5.

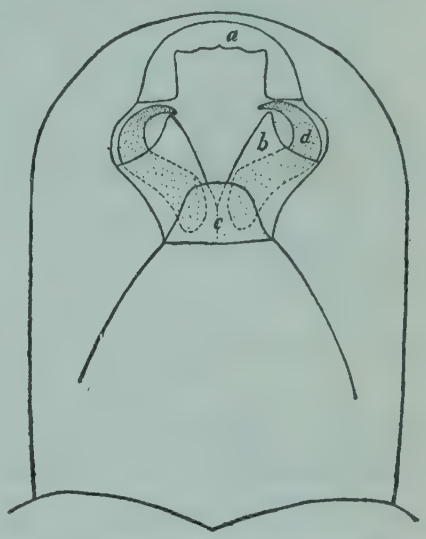


FIG. 4.—*Thersilochus conotracheli:* Ventral surface of head of first larval instar: *a*, Labrum; *b*, maxilla; *c*, labium; *d*, mandible. Highly magnified.

SECOND INSTAR.—With its first molt the larva acquires an entirely different appearance. The first larval skin splits longitudinally just back of the head and the forward part of the body is drawn out. Then the skin is pushed off from the caudal end of the body. The head shield remains intact. The larva that emerges lacks the taillike appendage and the prominent, heavily chitinized head. Its mouth parts are apparently entirely soft, and the most careful preparation and mounting of specimens has failed to disclose any mandibles. The mouth has the appearance of a dimplelike depression without armature. At full growth this instar (fig. 6) is about 3 mm. long. The head measures 0.31 mm. broad.

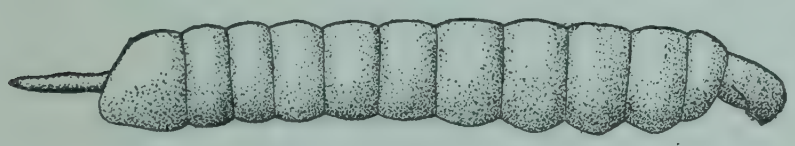

FIG. 5.—*Thersilochus conotracheli:* Full grown larva of the first instar. Highly magnified.

THIRD INSTAR.—The third instar is very like the second, except that it is larger, slightly stouter, and the head is 0.38 mm. broad. This measurement constitutes the only infallible distinction between the two stages. At full growth the third instar is about 3.50 mm. long.

FOURTH INSTAR.—With the assumption of the fourth instar the larva acquires the typical ichneumonoid larval characteristics. It is now in the form of a curved spindle, thick in the middle and tapering toward each end. It is about 4 mm. long with the head nearly a half millimeter broad, and with fairly distinct mouth parts. With high magnification the labrum, mandibles, maxillæ, and labium can be distinguished as well as the maxillary and labial palpi. The palpi appear merely as low rounded elevations on the surface of the maxillæ and labium. The mandibles are cone-shaped, and somewhat drawn out to

FIG. 6.—*Thersilochus conotracheli:* Larva of second instar. Greatly enlarged.

an acute, fairly strongly chitinized point. They are about 0.06 mm. long. The fourth molt takes place when the larva is about 4.75 mm. long.

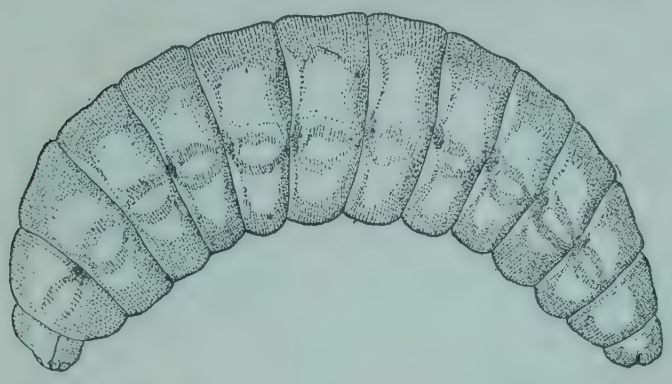

Fig. 7.—*Thersilochus conotracheli:* Full-grown larva, or fifth instar. Much enlarged.

FIFTH INSTAR.—The fifth instar (fig. 7) is a somewhat enlarged replica of the fourth, with the mandibles and other mouth parts more heavily chitinized and somewhat larger. The mandibles are of nearly the same form as those of the fourth instar, but are about 0.074 mm. in length. In this instar also the ridges supporting the mouth parts are strongly chitinized and can be faintly seen through the skin, especially the one extending from the base of the mandible around to the sides of the head. (See fig. 8.) Immediately after the molt this instar is about 5 mm. long, and at full growth, after it has left the body of its host and entirely consumed the fluid contents, it has reached a length of about 7 mm.

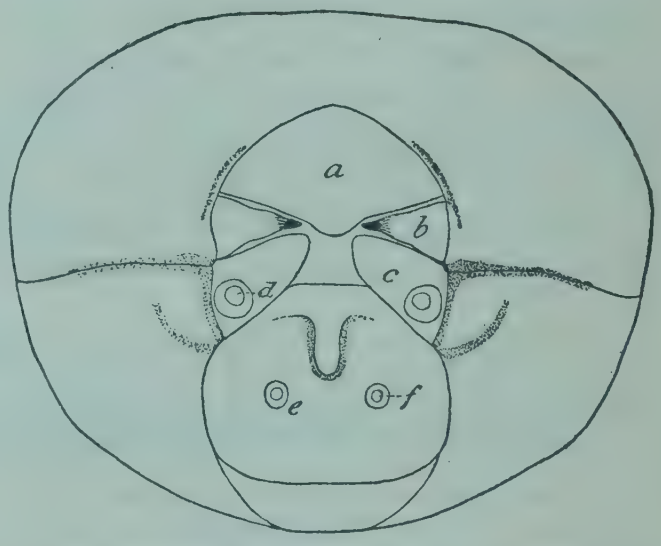


FIG. 8.—*Thersilochus conotracheli:* Face of full-grown larva: *a*, Labrum; *b*, mandible; *c*, maxilla; *d*, maxillary palpus; *e*, labium; *f*, labial palpus. Highly magnified.

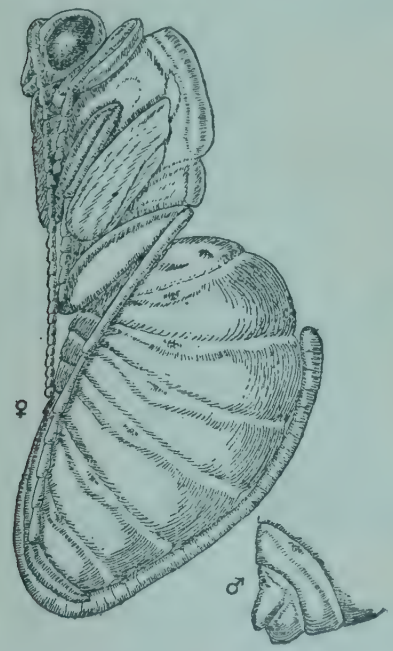


FIG. 9.—*Thersilochus conotracheli:* Pupa of female, and apex of abdomen of male pupa. Much enlarged.

## THE PUPA

The pupa (fig. 9) is stout, about 4.5 mm. long, and in the female has the ovipositor curved up over the back and reaching about two-thirds of the way to the thorax. The abdomen is about two-thirds as deep as long and is much stouter than and nearly twice as long as the thorax. The thorax is blocky, with the rather small head situated near the ventral anterior margin. The antennæ reach to about the middle of the abdomen and the hind legs nearly to the apex. The abdomen of the male pupa is terminated by three lobes, one dorsal and two ventral, the latter representing the genital armature of the adult.

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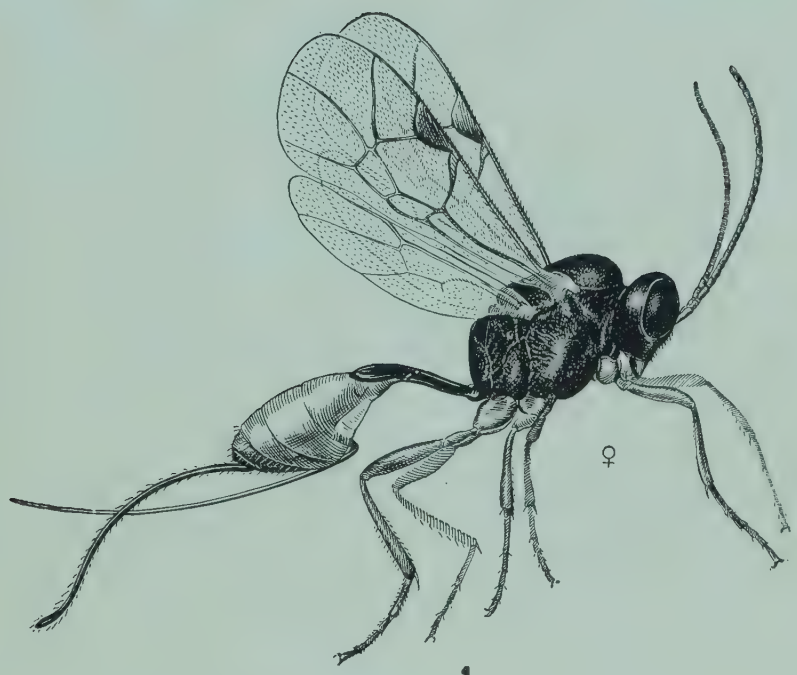
## PLATE CIX

*Thersilochus conotracheli:*

Fig. 1.—Adult female. Greatly enlarged.
Fig. 2.—*a*, Adult male; *b*, side view of abdomen. Greatly enlarged.

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# EFFECT ON PLANT GROWTH OF SODIUM SALTS IN THE SOIL

By FRANK B. HEADLEY, *Superintendent, Truckee-Carson Field Station*, E. W. CURTIS, *Scientific Assistant, and* C. S. SCOFIELD, *Agriculturist in Charge, Office of Western Irrigation Agriculture, Bureau of Plant Industry*

## INTRODUCTION

In connection with an attempt to utilize for crop production certain salty land on the Truckee-Carson Field Station, at Fallon, Nev., it has been necessary to make numerous determinations as to the limit of the salt content of the soil tolerated by crop plants. These determinations have shown that this limit of tolerance is not a fixed and definite point, but is instead extremely variable. Not only is it influenced by many factors, such as kind of soil, kind of salt, and kind of plant, but the same crop plant shows marked differences in tolerance at different periods of its growth. These facts make the problem of dealing efficiently with the reclamation of alkali land a very complex one.[1]

In the present instance the more abundant and deleterious salts are those of sodium. These sodium salts occur as carbonates, bicarbonates, chlorids, and sulphates, and the proportions of each in different parts of the field are extremely variable. This variability of the proportions in which these salts occur confused the results of the early attempts to determine the limits of tolerance for the different crops. In order to establish a basis from which to proceed with the work, a series of pot cultures was carried on in which the soils were artificially impregnated with solutions of the different salts. These experiments have served to show the limit of tolerance to each of the four salts of one crop, wheat, in the seedling stage. They have also brought out a point which has not generally been taken into account in similar experiments—that the limit of tolerance of plants is dependent not upon the quantity of salt added to the soil but upon the quantity which exists in the soil solution and which is recoverable from the soil by water digestion.

It appears that the discrepancy between the amount of salt added to a soil and the amount which can be later recovered from it is sometimes very great. Different soils show different results in this respect; and some of the salts, particularly the carbonates and sulphates of sodium, are absorbed by the soil to a greater extent than sodium chlorid. Thus, if the limit of tolerance of a plant is given in terms of the quantity of salt which must be added to a soil to inhibit growth, this limit will be found

[1] For literature germane to this subject see Harris, F. S. Effect of alkali salts in soils on the germination and growth of crops. *In* Jour. Agr. Research, v. 5, no. 1, p. 52–53. 1915.

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to differ from one given in terms of the salt recoverable from the same soil. In actual field practice salt lands must be classified in terms of the amount of salt recoverable from them and not in terms of the amount which has been added to them, which is not ascertainable.

## PLAN OF THE EXPERIMENTS

The general plan of all of the experiments was as follows: Ordinary drinking glasses were filled with 300 gm. of air-dry soil. The salts were added from a stock solution of known strength and ranged in amount from nothing up to concentrations sufficiently strong to prevent plant growth entirely. Distilled water was added to each glass to moisten the soil thoroughly. Seven seeds of wheat (*Triticum* spp.) were planted in each glass, and after germination the number of plants was reduced to five if more than that number came up. Bluestem wheat was used in 1913 and 1914, and Marquis wheat in 1915. To prevent loss of moisture, the glasses were covered with glass plates until the plants emerged. After the emergence of the plants, the pots were weighed daily, and by the addition of distilled water the moisture content was brought back to the original condition. In 1913 and 1914 the experiments were conducted in triplicate, while in 1915 they were in duplicate.

The wheat was allowed to grow from 15 to 18 days, when the plants were cut at the surface of the ground and weighed immediately in a closed tube. After cutting the plants the soils from each series of glasses were mixed, dried, and analyzed for water-soluble salts.

## CONVERSION OF CARBONATES

In the experiments where sodium carbonate was added to the soil, the analysis of the soil after the wheat had been cut brought out the fact that a portion of the sodium carbonate that had been added was not recoverable.

When only a small quantity of sodium carbonate was added, none could be recovered at the end of the experiment, but the quantity of sodium bicarbonate was greater than in the untreated soil. With the addition of larger quantities of sodium carbonate both salts were recovered at the end of the experiment, but their sum was always less than the quantity added at the beginning.

It is apparent that a portion of the sodium carbonate added to the soil was converted into sodium bicarbonate. In order to determine what proportion of the original quantity of sodium carbonate could be accounted for at the end of the experiment, it was necessary to add together the quantity of sodium carbonate recovered as such and the quantity represented in the form of sodium bicarbonate.

The conversion of sodium carbonate to sodium bicarbonate results in an increase in weight of the salt at the ratio of 44 to 70—that is, the

weight of a quantity of sodium carbonate is 63 per cent of the weight of the sodium bicarbonate that could be formed from it.

In the following tables and discussions the sum of the sodium carbonate and 63 per cent of the sodium bicarbonate found in the soil solution have been designated as "carbonate salts."

## EFFECT OF SODIUM CARBONATE ON WHEAT SEEDLINGS

EXPERIMENT 1.—The soil used in this experiment was obtained on the farm of the Truckee-Carson Field Station. It would be classed as a fairly productive sandy loam. It was analyzed for alkali salts and found to contain but a small quantity. The samples were made up in triplicate and sodium carbonate in solution was added to each set in the following percentages to the dry weight of the soil: Series 1, no treatment; series 2, 0.05; 3, 0.10; 4, 0.15; 5, 0.20; 6, 0.25; 7, 0.30; 8, 0.35; 9, 0.40; 10, 0.45; 11, 0.50; 12, 0.60.

Wheat was planted on November 1, 1913, and cut and weighed on December 11. Because of the lateness of the season, the growth had been very slow. After the wheat seedlings were removed, the soil from each set of pots was composited for analysis. The analysis was made of the solution secured by thorough digestion with an excess of water. The condensed results of this experiment are given in Table I.

TABLE I.—*Results of experiment 1 (1913), giving the quantity of sodium carbonate added to the soil, the quantity finally recovered as carbonates, the number of plants, and the combined weight produced in each case*

| Series No. | Sodium carbonate. | | Sodium bicarbonate recovered from soil.[1] | Total carbonate salts recovered. | Number of plants. | Green weight of plants. | | | | Decrease in yield from check pot. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Added to soil. | Recovered from soil. | | | | Pot 1. | Pot 2. | Pot 3. | Total. | |
| | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* | | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Per ct.* |
| 1 | 0 | 0 | 0 | 0 | 15 | 0.973 | 1.009 | 0.937 | 2.919 | ..... |
| 2 | .05 | 0 | .02 | .013 | 15 | .978 | .950 | .974 | 2.902 | 0 |
| 3 | .10 | Trace. | .04 | .025 | 15 | .697 | .865 | .847 | 2.409 | 17 |
| 4 | .15 | .02 | .04 | .045 | 13 | .619 | .061 | .116 | .796 | 73 |
| 5 | .20 | .03 | .05 | .062 | 10 | .220 | .055 | .044 | .319 | 89 |
| 6 | .25 | .07 | .05 | .102 | 5 | .008 | .012 | .020 | .040 | 98.7 |
| 7 | .30 | .11 | .02 | .123 | 1 | 0 | .008 | 0 | .008 | 99.7 |
| 8 | .35 | ..... | ..... | ..... | 0 | 0 | 0 | 0 | 0 | 100 |

[1] In excess of sodium bicarbonate present at beginning of experiment.

This experiment showed that where more than 0.30 per cent of sodium carbonate was added to the soil no plant growth was obtained; therefore the analyses of the higher percentages have not been included in Table I. The addition of 0.15 per cent of the salt reduced the germination of the seed, so that the full number of plants was not obtained and the total green weight produced was 73 per cent below that of the check series.

Beyond this critical point the reduction of germination and growth was rapid and consistent.

The experiment also showed that the quantity of sodium carbonate recoverable at the end of the experiment was much less than had been added to the soil at the beginning. The quantity of sodium bicarbonate had been increased in every case, but the total carbonate salts recovered was much less than had been added.

The apparent loss of the sodium carbonate added to the soil is shown in the difference between the figures in columns 2 and 5 of the table. The data of Table I are shown graphically in figure 1.

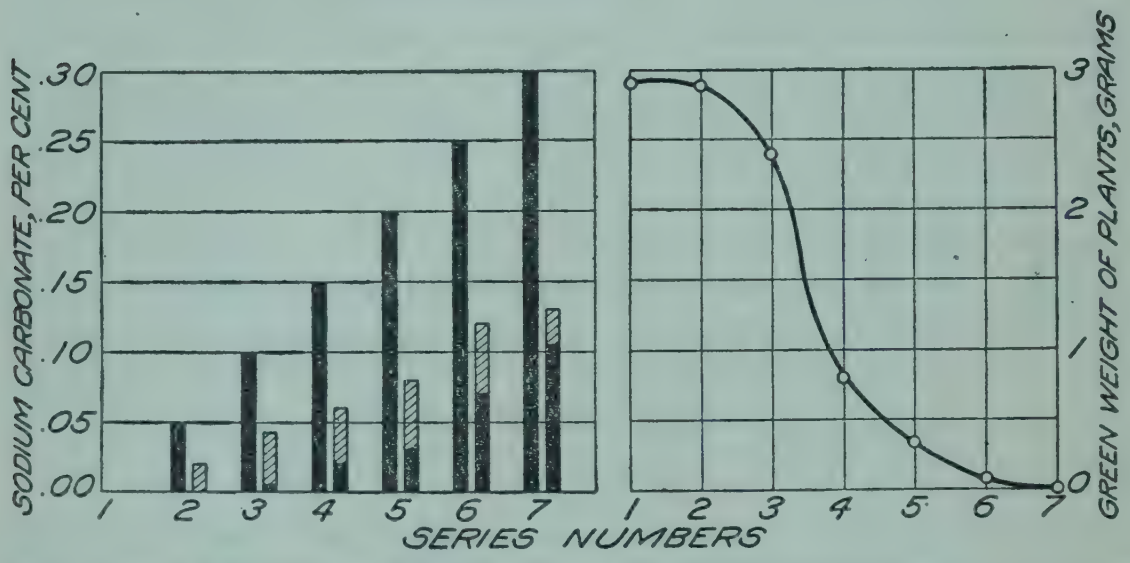


FIG. 1.—Diagram of the percentage of sodium carbonate added to the soil in experiment 1 (1913), with the percentage of carbonate and bicarbonate recovered and the total green weight of wheat obtained. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

EXPERIMENT 2.—The experiment was in most respects a repetition of experiment 1. The soil used was also taken from the same farm and was of the same physical character, but had been made more productive by the use of farm manure on the field from which it was taken. A preliminary analysis of this soil gave the following results: Sodium carbonate, 0; sodium bicarbonate, 0.091 per cent; sodium chlorid, 0.006 per cent; sodium sulphate, 0.

A triplicate series of sample pots were made up as before and sodium carbonate in solution was added as shown in Table II.

The wheat was planted in the pots on September 22, 1914, and cut and weighed on October 5. It was noted that with the successive increases in the percentage of carbonate added the time required for germination was increased, the percentage of germination decreased, and the amount of growth, both of leaves and roots, decreased. The results of this experiment are summarized in Table II.

TABLE II.—*Results of experiment 2 (1914), giving the quantity of sodium carbonate added to the soil, the quantity finally recovered as carbonates, the number of plants, and the combined weight produced in each case*

| Series No. | Sodium carbonate. | | Sodium bicarbonate recovered from soil.[1] | Total carbonate salts recovered. | Number of plants. | Green weight of plants. | | | | Decrease in yield from check pot. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Added to soil. | Recovered from soil. | | | | Pot 1. | Pot 2. | Pot 3. | Total. | |
| | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* | | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Per ct.* |
| 1 | 0 | 0 | 0 | 0 | 15 | 1.013 | 0.951 | 1.102 | 3.066 | 0 |
| 2 | .05 | 0 | 0 | 0 | 15 | 1.025 | .966 | .778 | 2.769 | 10 |
| 3 | .10 | 0 | .03 | .019 | 12 | .697 | .776 | .554 | 2.027 | 33 |
| 4 | .15 | .02 | .03 | .039 | 11 | .551 | .680 | .450 | 1.681 | 45 |
| 5 | .20 | .03 | .03 | .049 | 6 | .450 | .127 | .327 | .904 | 71 |
| 6 | .25 | .04 | .04 | .065 | 6 | .064 | .115 | .281 | .460 | 85 |
| 7 | .30 | .06 | .04 | .085 | 3 | .028 | 0 | .145 | .173 | 94 |
| 8 | .35 | .07 | .05 | .102 | 3 | .044 | 0 | .273 | .317 | 90 |
| 9 | .40 | .12 | .03 | .139 | 1 | .020 | 0 | .059 | .079 | 97.5 |

[1] In excess of sodium bicarbonate present at beginning of experiment.

The results of experiment 2 are in close accord with those of No. 1, although the decrease in yield was not quite so rapid. The apparent loss of carbonates—that is, the difference between the amount added and that recovered—was slightly greater. It is noticeable that in both experiments the percentage of sodium bicarbonate recoverable did not increase materially with the percentage of carbonate added, while there was a fairly consistent increase in the percentage of carbonate recovered. Furthermore, it will be observed that the decrease in yield follows the increase in total carbonates recovered more closely than the increase in carbonate added to the soil.

The results of experiment 2 are shown graphically in figure 2, in which the same arrangement of symbols is used as in figure 1.

EXPERIMENT 3.—This experiment was undertaken for the purpose of comparing the toxic effect of sodium carbonate on the growth of wheat seedlings in two very different types of soil. The first of these was a rich loam soil from an old alfalfa field on the Truckee-Carson Irrigation Project, and the second was beach sand obtained from Monterey, Cal. The experiment was conducted in the summer of 1915. Duplicate sets of pots were used in each case. After the salt had been added to the pots, the moisture content was kept at 12 per cent in the sand and 15 per cent in the loam. This arrangement had the disadvantage of making the concentration of the soil solution different in the two soils, but it was considered necessary because 15 per cent of moisture in the sand would have kept it too wet, and less than 15 per cent in the loam soil would not have been sufficient for the best growth of the plants.

The difference in the effect of the carbonate in the two soils was evident in a very few days. The time required for the wheat to emerge was approximately the same in both cases, but in the sand all germination was stopped by the addition of 0.20 per cent of sodium carbonate, whereas it required the addition of 0.50 per cent to the loam to have the same effect. The carbonate had a very detrimental effect on the physical condition of the loam soil, causing a stiff crust to form on the top of the pots, the crust becoming more noticeable with the increase of the percentage of carbonate. This made it difficult for the plants to break through. The detailed results of the experiment with loam and sand soils are given in Table III.

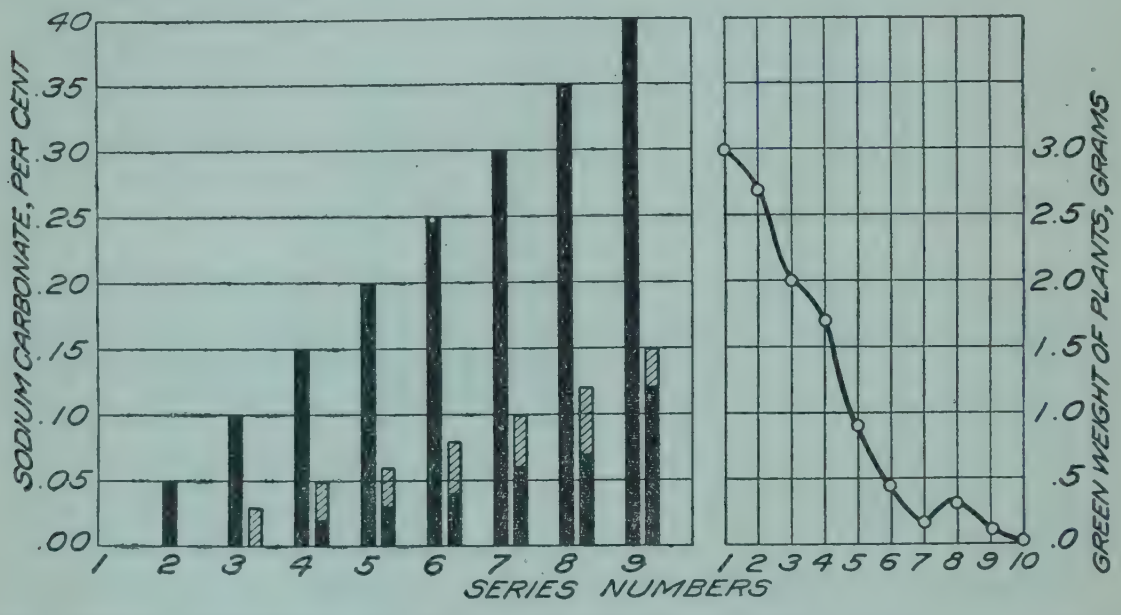


FIG. 2.—Diagram of the percentage of sodium carbonate added to the soil in experiment 2 (1914), with the percentage of carbonate and bicarbonate recovered and the green weight of wheat obtained. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

A comparison of the data on loam and Monterey sand (Table III) shows that the decrease in the yield of the plants was much more rapid in the sand than in the loam. The apparent loss of carbonates was much greater in the loam than in the sand. The loam soil also showed a steady increase in the amount of recoverable sodium bicarbonate, which was not the case with the sand.

A marked difference is to be noted in the green weight of the plants grown in the loam and in the sand. In the check pots the green yield from the sand series was only 62 per cent of the yield of the plants in the loam, although the average height of the plants in the two series was approximately the same.

The data presented in Table III are shown graphically in figures 3 and 4.

TABLE III.—*Results of experiment 3 (1915), giving the effect of sodium carbonate in loam soil and in Monterey sand on the germination and growth of wheat*

LOAM SOIL

| Series No. | Sodium carbonate. | | Sodium bicarbonate recovered from soil.[1] | Total carbonate salts recovered. | Number of plants. | Green weight of plants. | | | Decrease in yield from check pot. |
|---|---|---|---|---|---|---|---|---|---|
| | Added to soil. | Recovered from soil. | | | | Pot 1. | Pot 2. | Total. | |
| | *Per ct.* | *Per ct.* | *Per cent.* | *Per cent.* | | *Gm.* | *Gm.* | *Gm.* | *Per ct.* |
| 1 | 0 | 0 | 0 | 0 | 10 | 0.791 | 0.700 | 1.491 | 0 |
| 2 | .05 | 0 | .03 | .019 | 10 | .725 | .782 | 1.507 | 0 |
| 3 | .10 | 0 | .05 | .032 | 10 | .535 | .708 | 1.243 | 17 |
| 4 | .15 | 0 | .04 | .025 | 10 | .588 | .629 | 1.217 | 19 |
| 5 | .20 | 0 | .05 | .032 | 10 | .666 | .520 | 1.186 | 21 |
| 6 | .25 | 0 | .08 | .050 | 9 | .497 | .420 | .917 | 38.5 |
| 7 | .30 | 0 | .10 | .063 | 8 | .255 | .538 | .793 | 47 |
| 8 | .35 | 0 | .16 | .101 | 5 | 0 | .351 | .351 | 76 |
| 9 | .40 | .008 | .18 | .122 | 4 | .186 | .077 | .263 | 83 |
| 10 | .50 | .013 | .21 | .145 | 0 | 0 | 0 | 0 | 100 |
| 11 | .60 | .023 | .22 | .162 | 0 | 0 | 0 | 0 | 100 |

MONTEREY SAND

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 10 | .486 | .445 | .931 | 0 |
| 2 | .05 | .003 | .066 | .045 | 10 | .176 | .176 | .352 | 62 |
| 3 | .10 | .013 | .087 | .068 | 6 | .069 | .030 | .099 | 89 |
| 4 | .15 | .016 | .119 | .091 | 5 | .027 | .024 | .051 | 96 |
| 5 | .20 | .049 | .132 | .132 | 0 | 0 | 0 | 0 | 100 |
| 6 | .25 | .080 | .116 | .153 | 0 | 0 | 0 | 0 | 100 |
| 7 | .30 | .096 | .125 | .175 | 0 | 0 | 0 | 0 | 100 |
| 8 | .35 | .140 | .137 | .226 | 0 | 0 | 0 | 0 | 100 |
| 9 | .40 | .244 | .159 | .344 | 0 | 0 | 0 | 0 | 100 |

[1] In excess of sodium bicarbonate in soil at beginning of experiment.

## THE EFFECT OF SODIUM BICARBONATE ON WHEAT SEEDLINGS

EXPERIMENT 4.—In order to determine the relative toxicity of the carbonate salts when added to a soil in the form of sodium bicarbonate, experiment 4 was undertaken in the summer of 1914. The technique of this experiment was the same as that of the experiments previously described, the series of pots being triplicated. The soil used was of the same type as that in experiment 2. The range of salts added to the soil was greater than in the first two experiments, including series numbered 8, 9, and 10, in which were added 0.80, 1, and 1.25 per cent of sodium bicarbonate. Since there was no germination or growth in these series, they have not been included in Table IV, which gives a summary of the results of the experiment.

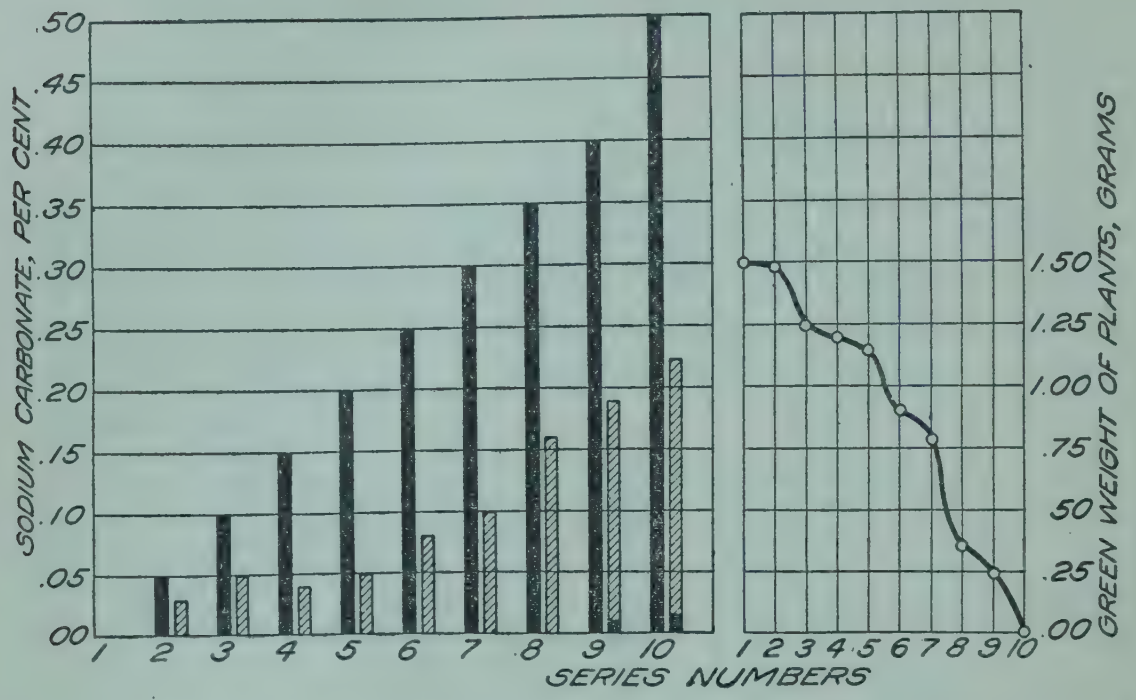


FIG. 3.—Diagram of the percentage of sodium carbonate added to the loam soil in experiment 3 (1915), with the percentage of carbonate and bicarbonate recovered, and green weight of wheat. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

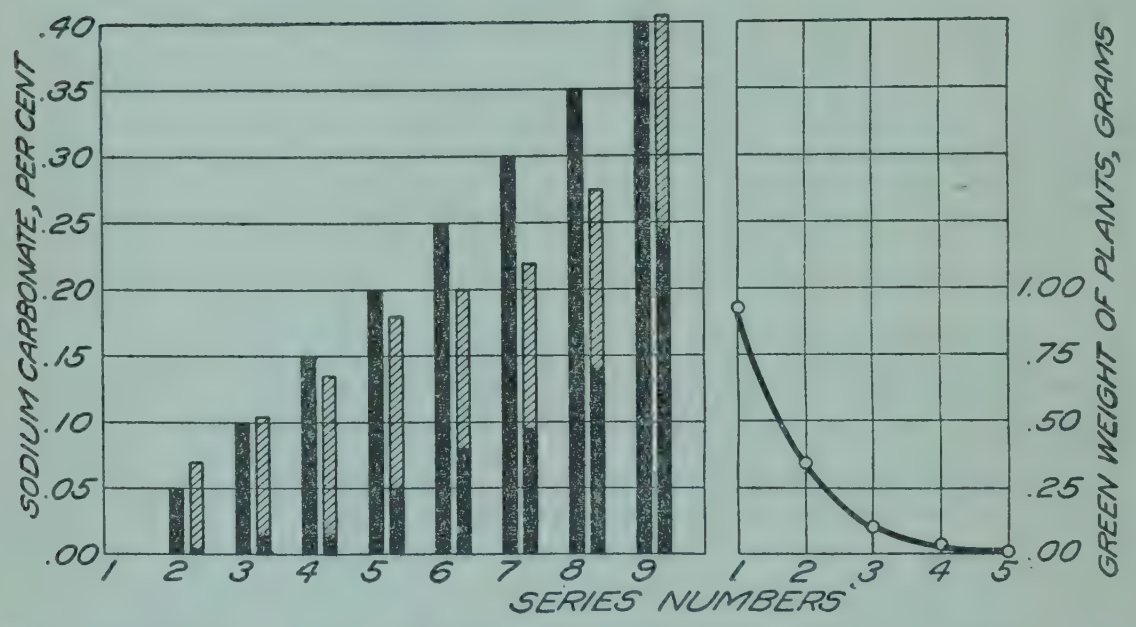


FIG. 4.—Diagram of the percentage of sodium carbonate added to Monterey sand in experiment 3 (1915), with the percentage of carbonate and bicarbonate recovered and the total green weight of wheat obtained. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

TABLE IV.—*Results of experiment 4 (1914), giving the effect of sodium bicarbonate on the germination and growth of wheat*

| Series No. | Sodium bicarbonate. | | Sodium carborate recovered from soil. | Total carbonate salts recovered. | Number of plants. | Green weight of plants. | | | | Decrease in yield from check pots. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Added to soil. | Recovered from soil. | | | | Pot 1. | Pot 2. | Pot 3. | Total. | |
| | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* | | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Per ct.* |
| 1 | 0 | 0 | 0 | 0 | 15 | 0.996 | 0.917 | 1.032 | 2.945 | 0 |
| 2 | .05 | 0 | 0 | 0 | 15 | 1.051 | .790 | .905 | 2.746 | 6.8 |
| 3 | .10 | .012 | 0 | .008 | 15 | .856 | .841 | .929 | 2.626 | 10.8 |
| 4 | .20 | .029 | 0 | .018 | 15 | .981 | .873 | .819 | 2.673 | 9.2 |
| 5 | .30 | .071 | 0 | .045 | 15 | .362 | .460 | .371 | 1.193 | 59.5 |
| 6 | .40 | .088 | .021 | .076 | 5 | .065 | .114 | .070 | .249 | 91.5 |
| 7 | .60 | .071 | .074 | .119 | 0 | 0 | 0 | 0 | 0 | 100 |

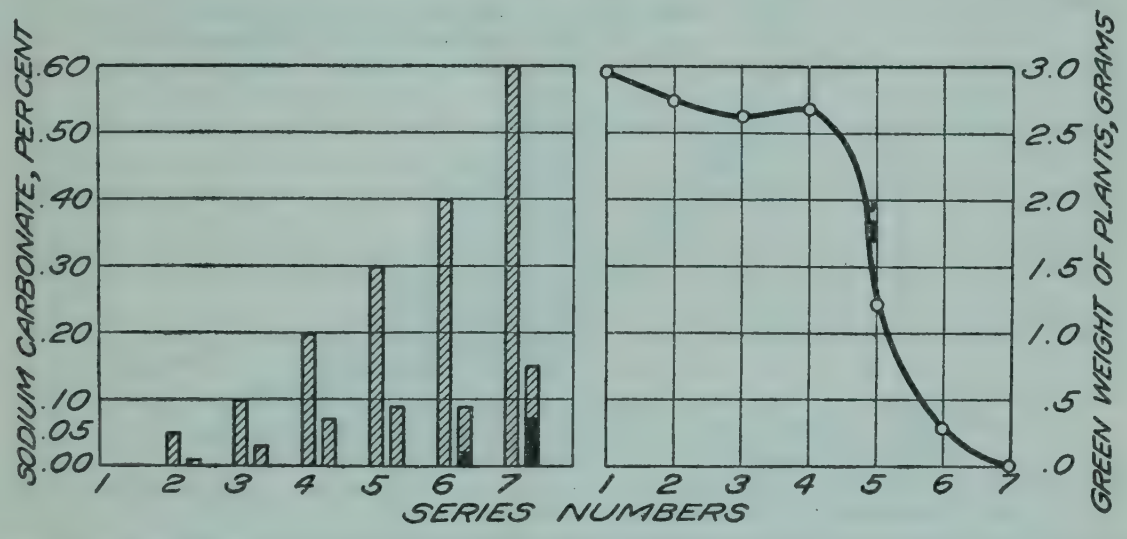


FIG. 5.—Diagram of the percentage of sodium bicarbonate added to the soil in experiment 4 (1914), with carbonate and bicarbonate recovered, together with the total green weight of wheat obtained. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

The first noticeable feature of this experiment is the discrepancy between the amount of bicarbonate added to the soil and the amount finally recovered. There was also evidence of a conversion of the bicarbonate to the carbonate form in the last two series. In this case, as in experiment 2, there was a decrease of growth in series 2, even though no carbonate salt was recoverable at the end of the experiment.

The data presented in Table IV is shown graphically in figure 5.

## COMPARATIVE TOXICITY OF SODIUM CARBONATE AND SODIUM BICARBONATE

A comparison of experiments 2 and 4, in which the carbonate and bicarbonate salts were used, shows that these two salts have approxi-

mately the same toxic effect when the total of the carbonate salts recoverable is considered rather than the percentage of salts added to the soil. In other words, the toxicity of these salts in the soil is directly associable with the quantity of the basic radical in the salt recoverable. The close relationship between the results of these two experiments is shown in figure 6, in which the curves of decrease in growth are constructed on the same scale.

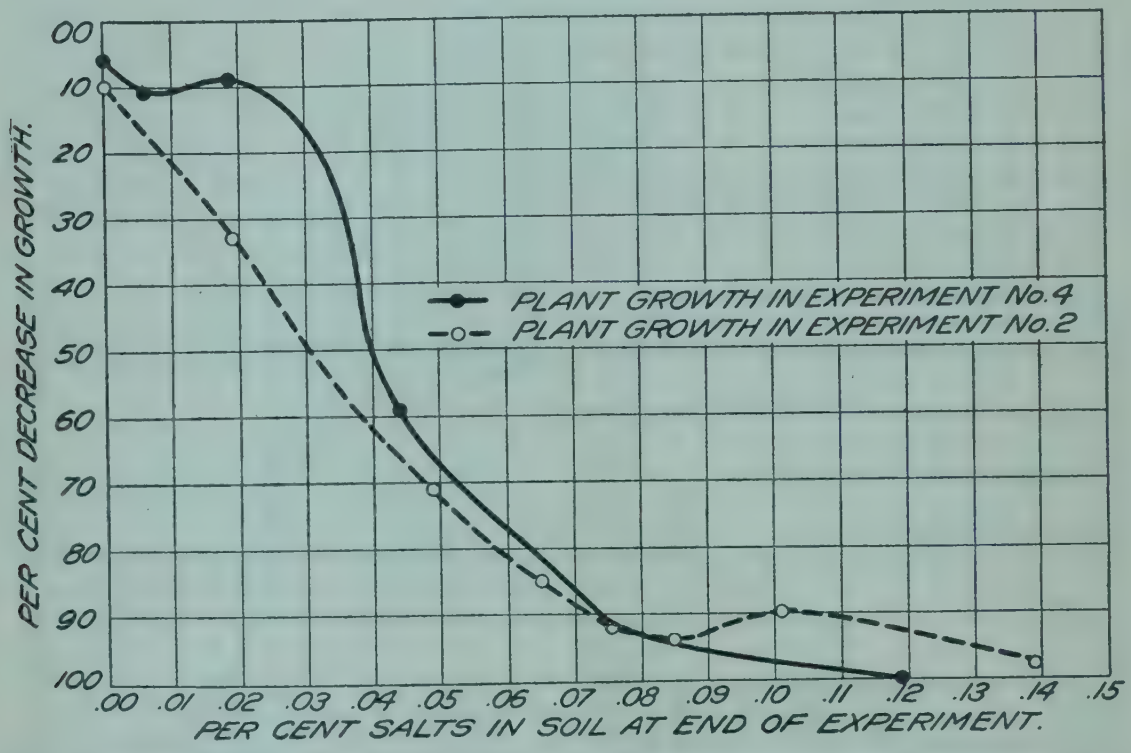


FIG. 6.—Diagram of the decrease in growth of wheat seedlings in experiments 2 and 4 as affected by the total carbonate salts recoverable from the soil. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

## EFFECT OF SODIUM CHLORID ON WHEAT SEEDLINGS

EXPERIMENT 5.—At the same time the carbonate and bicarbonate experiments in 1914 were in progress (experiments 2 and 4) a similar experiment with sodium chlorid was carried on with the same soil. The general plan and manipulation was the same as has been described above. The wheat was allowed to grow for 16 days. The original soil contained only 0.006 per cent of sodium chlorid.

A summary of the results obtained in this experiment is given in Table V. It is possible to compare these results directly with those obtained in the carbonate, bicarbonate, and sulphate experiments in 1914, as the soil used was the same in all cases.

TABLE V.—*Results of experiment 5 (October, 1914), giving the effect of sodium chlorid in soil on the germination of wheat seedlings*

| Series No. | Sodium chlorid. | | Number of plants. | Green weight of plants. | | | | Decrease in yield from check pots. |
|---|---|---|---|---|---|---|---|---|
| | Added to soil. | Recovered from soil. | | Pot 1. | Pot 2. | Pot 3. | Total. | |
| | *Per ct.* | *Per ct.* | | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Per ct.* |
| 1 | ..... | ..... | 15 | 0.943 | 0.852 | 0.980 | 2.776 | ..... |
| 2 | 0.05 | 0.04 | 15 | .824 | .818 | .821 | 2.464 | 11.0 |
| 3 | .10 | .09 | 13 | .724 | .646 | .642 | 2.013 | 27.5 |
| 4 | .20 | .16 | 13 | .420 | .430 | .562 | 1.412 | 49.0 |
| 5 | .30 | .26 | 5 | .115 | .093 | .178 | .388 | 86.0 |
| 6 | .40 | .32 | 5 | 0 | .029 | .015 | .044 | 98.4 |
| 7 | .60 | .52 | 0 | 0 | 0 | 0 | 0 | 100.0 |

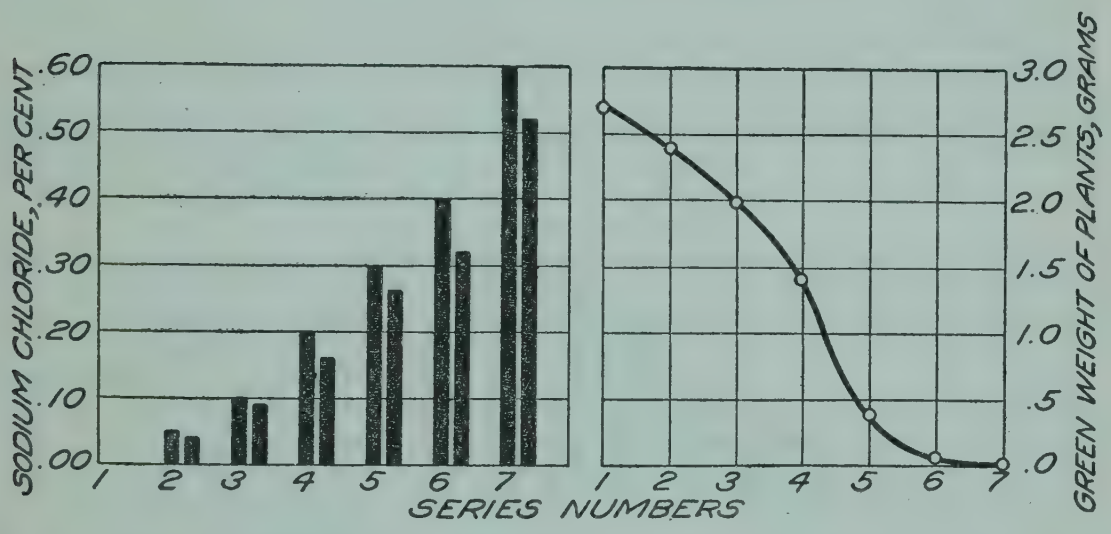


FIG. 7.—Diagram of the quantity of sodium chlorid added to the soil, with the quantity of chlorid recovered, and the total green weight of wheat obtained. Experiment 5. The left-hand column above the series number represents the percentage of sodium chlorid added and the right-hand column the percentage recovered from the soil. The curve at the right represents the weight (in grams) of the green wheat.

It is clear from the results given in Table V that the absorptive power of the soil for sodium chlorid is much less than for sodium carbonate. An average of 85 per cent of the chlorid was recovered, and a 50 per cent decrease in yield took place in the soil from which 0.16 per cent of the salt was recovered.

The results of Table V are shown graphically in figure 7.

## EFFECT OF SODIUM SULPHATE ON WHEAT SEEDLINGS

EXPERIMENT 6.—The experiment with sodium sulphate was conducted in the same manner and at the same time and with the same lot of soil as experiments 2, 4, and 5. The moisture content of the soil was kept at 16 per cent and the wheat was allowed to grow for 16 days. The results are summarized in Table VI.

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# OBSERVATIONS ON THE LIFE HISTORY OF THE ARMY CUTWORM, CHORIZAGROTIS AUXILIARIS[1]

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## INTRODUCTION

The army cutworm (*Chorizagrotis auxiliaris* Grote) occupies a prominent place among the pests of staple crops in the Northwest. Serious outbreaks have occurred at irregular intervals, and more or less damage is done nearly every year. Our knowledge of the life history of the species has been very incomplete, and further facts are obviously needed. The purpose of the present article is to present the results of studies made during several years and particularly some observations made in 1915 on the egg-laying habits of the species, together with their bearing on the question of the number of broods in the annual cycle.

## SCIENTIFIC NAME OF THE SPECIES

In previous years the writer has repeatedly reared to the adult stage larvæ which had been taken in destructive colonies of this insect. Among these he has found forms corresponding with descriptions of *Chorizagrotis auxiliaris*, *C. agrestis*, and *C. introferens*. Moths reared from much-rubbed parents caught in the fall of 1915, as recorded below, when determined were found to include at least two of these forms. Gillette[2] states that he had found these forms occurring together in colonies in Colorado. For these reasons and because *C. auxiliaris* has priority, this name has been used to designate this insect in the present paper.

## PREVIOUS EXPERIMENTAL EFFORTS

The attempts of the writer to obtain information regarding the life history of this species, and particularly regarding the egg-laying habits, date back for several years, and a brief summary of these efforts may be

[1] Since completing this manuscript and just as it is about to be offered for publication, the writer received a copy of Strickland's excellent paper, which covers somewhat the same ground. (Strickland, E. H., The army cutworm, Euxoa (Chorizagrotis) auxiliaris Grote. Canada Dept. Agr. Ent. Branch, Bul. 13, 1916.)

[2] Gillette, C. P. Some of the more important insects of 1903. *In* Colo. Agr. Exp. Sta. Bul. 94 (Tech. Ser. 6), p. 6. 1904.

of benefit to any who have occasion to search for the eggs of other Noctuidae. On account of the irregular occurrence of the species, it has been quite impossible to depend upon getting a supply of living specimens for study when wanted, and a continuous effort in the search for the facts desired has been out of the question. In the nature of the case, instead of laying out and following a definite plan of study the successful pursuit of which would certainly lead to the results desired, it has been necessary to rely largely upon scattering observations made through several years as opportunity was afforded.

The first attempt to obtain the early stages was made in 1907, when, in the writer's first experience with an outbreak of the species, a large number of moths originating from larvæ which had been brought in during the fall, were reared during the winter in the insectary and were held in large Riley-type cages in order that they might have an opportunity to lay eggs. All of the moths died within a few days, and no eggs were laid. In the spring several hundred larvæ were brought in from grain fields and fed to maturity. The moths which emerged were left in the cages and given water and a variety of plants upon which to lay eggs. Again the moths died without laying eggs.

Several explanations for the failure suggested themselves. It was thought possible that the moths were not normal because of having been reared in confinement. A number of female moths were dissected for the purpose of examining the ovaries, and it was found that the ova were immature. The idea suggested itself that the failure to develop ova was the outworking of some little understood principle of periodicity in the occurrence of the species, and it was also thought that the absence of mature ova might be due simply to the lack of food after the emergence of the adults.

In the spring of 1910 there was a destructive colony of the larvæ 8 miles west of Bozeman, and plans were laid to recover a supply of the pupæ from the soil, allow them to emerge in confinement, and attempt to feed the adults on honey water to get them to grow ova. Our trip to the field to secure pupæ was not correctly timed, and the moths had already emerged. Accordingly, an attempt was then made to obtain moths by catching them at night near the same field. A special trap light was arranged, consisting of a wooden box 18 inches square and 12 inches high with an 8-inch circular hole in the top into which was inserted a large funnel-shaped tin with a 2-inch opening at the bottom. Above the funnel was placed an acetylene light from a stereopticon. This furnished a very strong light, and the night was favorable. The writer and an assistant went to the field, expecting to spend the night. They remained until between 12 and 1 o'clock, but only a few moths of any kind came, and none of *C. auxiliaris*. As no encouragement whatever was received, even though it was known that many thousands of

moths had emerged recently in the vicinity, the experiment was abandoned.

On numerous occasions attempts have been made to find moths in the field in the act of depositing eggs, either on crops or on vegetation on virgin sod. Much time has also been spent in searching for eggs on grasses, clover, alfalfa, stools of volunteer grain, and other cultivated plants. A few eggs of other noctuids have been found, but none of this species.

## CAGE EXPERIMENTS

In April and May, 1915, there occurred in Montana a widespread and very severe outbreak of the army cutworm. Moving armies of the larvæ were reported daily, and many thousands of acres of fall wheat were eaten off during April and May, so that it was possible to obtain a supply of the living insects for study. Plans were made for a twofold effort in connection with the outbreak. It was decided to install several large cages outdoors and by any means possible to obtain adult moths of both sexes, place them in the cages, and attempt to feed and keep them alive until they should lay eggs, correlating the observations made in these cages with notes from the field.

Three large cages 24 inches square by 40 inches high were installed on the lawn near the insectary. These are of fine-mesh brass screening and are fastened to the earth by a broad baseboard which is inserted in the soil. A large door fills one side and in this door is a smaller one, sufficiently large to permit the insertion of the hand. On the 24th of April 28 larvæ were placed in cage 2 and 53 in cage 3. These worms had been collected from two fields at Willow Creek, Mont. They were fed regularly, but did not do well. It has been repeatedly found that it is difficult to rear a large proportion of these caterpillars when fed in a body in one cage. For this reason in rearing record specimens the writer has adopted the method of feeding the caterpillars individually in tin boxes and by so doing has brought nearly every individual to maturity. None of these caterpillars pupated in the large cages.

On June 10 about 300 pupæ which had been taken from the soil in a field at Willow Creek were brought to Bozeman and placed in cages 1 and 2. Not one of these emerged. When examined, they were dead and decayed. They had been placed individually in holes in the soil with the anterior end uppermost—a method which has been used with dry soil with much success in indoor rearing. It is possible that rains had closed the holes and injured the pupæ by puddling the earth around them. On the 20th of June, 13 moths which had been reared in the insectary were placed in cage 1, and on July 17 about 50 moths which were captured out of doors at Willow Creek were divided between cages 1 and 2. It was hoped that some of these would grow ova and deposit eggs. Sponges saturated with honey water were placed in the cages daily and clover

blossoms were picked and put in fresh each day. A variety of plants in small pots were placed in the cages, and as some of these blossomed, it is probable that they would furnish more or less nectar on which the moths might feed. The general condition in these cages certainly more closely simulated complete liberty than could have been provided in the insectary.

The writer was at first much disappointed in the results obtained, for by repeated examinations of the vegetation which had been placed in the cages, he was unable to find any eggs. The moths lived on, however, and served a very valuable purpose in indicating that the normal life of the moth in the open might be much longer than had been thought. While the moths gradually died off during the summer, many were alive on August 16, and several were seen as late as September 21. A pair was seen in copulation on August 10. Since no eggs had been found in the cages, the fact that the moths lived on until so late also suggested that perhaps the period between the emergence of the adult and oviposition might be much longer than had been suspected. The writer therefore determined to look for the moths in the fields late in the summer.

After eggs had been found in the field, as recorded below, a very careful search on the soil in the cages was made, but without finding any eggs.

## FIELD OBSERVATIONS IN 1915

During the season of 1915 the writer had an especially good opportunity to make observations in the field in connection with various trips to different parts of the State to aid farmers in the control of the cutworm and to collect material for the cages at Bozeman.

All through the summer, since some of the moths were still alive in the cages, the moths in the field or any clue that might indicate the time and place of egg laying were looked for. No moths were seen, except in or near fields which had been damaged, and even there none could be found a few weeks after the emergence of the moths. On July 16 and 17, moths were seen in great abundance at Willow Creek and were found hiding under clods of earth in the grain fields. At this time the last of the moths were just emerging, and some pupæ could yet be found. The same field was visited again on August 3, but no living moths could be found. Since the previous visit there had been a heavy rain, and by turning over clods of soil, many dead moths were found which had been trapped there. Since moths had been found so abundantly in this locality on July 16 and 17, it was thought that eggs also might be found. A careful, extended search, however, revealed none. Another search for eggs and young cutworms was made in this locality on September 21, but was entirely without results.

On July 17, the headlights of an automobile were used in the evening in an attempt to attract the moths in the field, but without much success.

In the early part of the evening one moth was caught, but a violent storm came up, preventing further search.

From this time until late in September, as recorded below, no moths were seen out of doors either at Willow Creek or elsewhere in the State. An electric-light moth trap was kept in use on fair, warm nights at Bozeman all summer, but no moths of this species were captured until late in September. Gillette[1] mentions what he considers to be two broods of the moths, one occurring between April 16 and July 10 and the other between September 13 and October 12. Wolley-Dod [2] records the capture of *C. auxiliaris*, *C. introferens*, and *C. agrestis* in Alberta, Canada, in June and July and states that one specimen each of *C. introferens* and *C. agrestis* was captured on September 9. One individual of *C. agrestis* was captured on May 19. Gillette also points out that he had been unable to find fully developed ova in the females of the first brood, though hundreds were dissected and examined, while dissected specimens of the fall brood, almost without exception, contained fully formed ova. This observation is of much importance and has been verified by the present author as stated elsewhere. It gives strong support to the conclusions of the present paper regarding the number of broods in the annual cycle. It also indicated that the ova are developed on food obtained as an adult rather than as a larva.

## OBSERVATIONS ON THE EGG-LAYING HABITS OF THE SPECIES

On September 30 Assistant Entomologist J. R. Parker, of the Montana Station, while out on the college farm, saw noctuid moths flying in fair abundance. One was captured and brought in. On close examination it turned out to be a much-rubbed female of *C. auxiliaris*. Mr. Parker and the writer returned to the field at once to watch the moths. They were laying eggs in abundance directly upon the soil—not on plants, as had been expected. During the next few days extended definite observations on the egg-laying habits of the species were made on the college farm.

Several pieces of land had been recently plowed and harrowed. One field of 10 to 12 acres had been particularly well prepared for seeding some days earlier and was nearly free from vegetation, though a few grains and weed seedlings and grasses were to be found. The moths were seen in abundance on the soil in this field in fair weather day after day.

Egg laying was confined to the warm afternoons, and the moths were most active in the latter part of the day, from 3 o'clock until sunset. The mornings in October in Bozeman are generally quite cold, but a few warm forenoons occurred during which an unsuccessful attempt was made to observe egg laying in the field. By looking toward the west into

[1] Gillette, C. P. Some of the more important insects of 1903. *In* Colo. Agr. Exp. Sta. Bul. 94 (Tech. Ser. 6), p. 6. 1904.

[2] Wolley-Dod, F. H. Preliminary list of the Macro-Lepidoptera of Alberta, N.-W. T. *In* Canad. Ent., v. 37, no. 2, p. 49. 1905.

the sun's rays during the late afternoon many moths could be seen flying or walking along the surface of the soil. The moths were repeatedly seen to fly into the field from the grasslands or stubble fields adjoining and stop in the tilled field, where they immediately began to lay eggs. Several times they laid eggs on the bare earth of the roads on the college farm.

The moths also laid eggs in one field on the college farm just after it was plowed. Not once was a moth seen to lay eggs on any green plant or in any green field or stubble field; nor were any eggs found in such fields. Again and again, while watching the moths laying eggs at close range in the tilled field, they were seen to pass close by different kinds of vegetation without pausing. It was perfectly evident that they preferred to lay the eggs in the soil. By being careful one could witness the egg laying in detail by following along on hands and knees as a moth alternately paused to lay eggs and walked for a short distance. By far the greater number of the eggs were placed on the surface of the soil, often on small clods of earth, the moth standing on the clod and bending the abdomen downward and often tucking the eggs on the underside of the clod. Generally one or two eggs were laid on one spot, a few seconds being taken for the process. Sometimes but not always the moth frisked the tip of the abdomen back and forth sidewise repeatedly across the spot where the eggs were laid, thus dusting them and leaving a few scales from the clothing of the insect. The bright, glistening-white eggs are thus obscured. Some of the eggs were laid just beneath the surface of the soil. This could be done only where the soil had been pulverized, and in accomplishing it the ovipositor is thrust down through the surface of the soil and left for a few seconds. It is difficult to find such eggs under the surface of the soil, even when the spot is seen and the examination is made at once. One egg was found on a piece of dead straw. Generally only one to three were laid in one place, but in one case a moth deposited many eggs in soft soil within the space of a few square inches.

From the number of moths seen on the field and the number of days egg laying continued, it was roughly estimated that at least one or two eggs per square foot were laid in this field. By carefully searching a spot selected at random, eggs, almost certainly of this species, could be found. Four different persons, including the writer, have found eggs on the soil without having seen the moths deposit them. Both sexes were found among the moths captured in the field during the period of egg laying.

From these observations it can not be said that the eggs are necessarily always laid on bare or broken soil. In fact, it is almost certain that they are sometimes laid in abundance where newly plowed or newly harrowed soil is not available. A field of alfalfa badly infested with these cutworms was seen in Utah by the writer in May of the present year (1916), but no soil that could have been plowed last fall was anywhere in the vicinity. However, the stand of alfalfa was very scattering, leaving

much bare soil between the plants. A much-traveled road near by probably did not account for the presence of the worms. It seems more likely that the eggs were laid on the bare patches of soil in the alfalfa field.

There can be no doubt as to the specific identity of the moths that were observed, for many taken in the act of egg laying were carried into the insectary and placed in chimney cages, where they laid eggs in large numbers. Larvæ hatching from these eggs were reared to maturity, and the moths were obtained and all identified as belonging to this species. A few moths of other species were seen in the field during these observations, but none except *C. auxiliaris* were seen to deposit eggs.

## ATMOSPHERIC TEMPERATURE DURING OVIPOSITION

No very definite temperature limits to oviposition can be stated. Thermometers were taken into the field and observed from time to time as the moths were being watched. The temperature during the rapid oviposition generally ranged between 55° and 70° F. A temperature of 60° to 70° at 4 or 5 o'clock p. m., with little or no wind stirring, insured great activity of the moths. When the sun set, the temperature dropped rapidly, and the moths sought shelter under clods and in cracks in the soil, very few being found still active in a temperature between 45° and 50°. Egg laying ceased at about 40°, though the moths were seen to fly at lower temperatures if disturbed. Whether or not the moths would continue active after dark if the temperature were favorable can not be stated.

## DESCRIPTION OF THE EGG

Viewed from above, the egg is circular in outline; but when viewed from the side, it is very nearly elliptical, the shape varying from an ellipse only by being slightly flattened on the side on which it rests, which is opposite the micropile. It measures 0.62 mm. in diameter by 0.52 mm. in height. The color when the egg is first laid is white tinged with yellow, but before hatching the dark embryo shows through, giving the effect of a darker color. Surface markings on the chorion are very obscure. They are invisible under a hand lens magnifying 16 diameters. When viewed by reflected light under a compound microscope or under the high power of a binocular microscope, a very faint reticulation may be seen. This is more distinct in the shells of hatched eggs, in which the pattern often may be very clearly seen. No ridges radiating from the apex or upper part of the egg, such as may often be seen in noctuid eggs, have been found in this species.

## EGG-LAYING HABITS IN CONFINEMENT

The moths taken into the insectary and put in cages were quite irregular in their egg laying. In general, two or three days were passed without laying eggs; then a large number were laid within a few hours, after which the moths soon died. Clover blossoms were placed in the cages,

and the moths were seen to be apparently feeding on the nectar. In the field during the same period the moths were seen to pause in their flight from field to field and visit blossoms of such plants as mustard and clover.

The various lots of eggs were allowed to hatch and the larvæ were reared in the greenhouse during the winter. Critical notes on instars and stadia were also made for later publication.

## DURATION OF THE EGG-LAYING PERIOD

It is not probable that the writer observed the very beginning of the egg-laying period when, on September 30, the first moths were seen to be laying. It is altogether probable that the period began some days earlier, and there is no absolute evidence that egg laying had not been in progress for some weeks. It is quite clear, however, that the period closed about October 14, when a cold spell with rain occurred. When the weather cleared again, observations were resumed in the fields, but only a few scattering moths could be found, even though the temperature was favorable. October 8 was noted as the date on which the maximum activity of moths and egg laying was observed, and some eggs were laid as late as October 12. It may be safely stated that the egg-laying period is two weeks or more in duration.

## NUMBER OF EGGS LAID

A detailed record of the number of eggs laid by individual moths was not made, as several circumstances in connection with the methods used interposed to make this difficult. The writer was influenced also by the fact that the moths had laid a part of their full number of eggs before being confined, which made it impossible to get complete data. The largest number actually counted was 252, all laid during one night, but this probably falls considerably below the actual maximum number. From this the number varies down to a very few, which may be accounted for in part by the moths having laid eggs in the field before being captured. The moth that laid 252 eggs died soon after and was dissected, the ovaries showing many immature ova.

## DURATION OF THE INCUBATION PERIOD

The writer has complete records of the duration of incubation in 23 lots of eggs. A part of these were kept in the insectary and a part in an outdoor shelter. The minimum period recorded is 9 days, the maximum 21 days, and the average 16.77 days. The wide variation shown is striking and can probably be explained. The egg lots were kept during incubation in small tin boxes which were opened and examined daily. It was observed that many eggs were badly shrunken, and the dark embryos could be seen through the chorion. It was decided to add a very small amount of water to each box, as it was feared that the eggs would die from dryness. Accordingly, 1 to 3 drops of water were placed

in each of the boxes and the eggs hatched within a few hours. It seemed to be clear that incubation had been completed some days earlier in some of the lots, but that the young caterpillars had been prevented from issuing until sufficient moisture was present. Thus, some hatched in 9, while others hatched in 21 days. Those in the outdoor shelter hatched as soon as those inside. It is probable that 9 or 10 days is about the correct incubation period.

No field data on the incubation period are available. Repeated searching revealed no newly hatched caterpillars in fields where numerous eggs were known to have been laid. It is very interesting to note also that no larvæ could be found this spring in the field on the college farm where the eggs are known to have been laid last fall.

## LARVAL FEEDING IN THE FALL

Only scattering records of larvæ in the fall are available, but these are of considerable interest. In the fall of 1906 the very small larvæ of this species did some damage in the northern part of Gallatin Valley. Several lots of the larvæ were received at the Experiment Station in November, and reports of their occurrence had reached it in October. One lot was reared to the adult condition. This is the only case known here in which the larvæ have attracted the attention of the farmers in the fall, and in this case the knowledge of their presence served as a useful warning of their coming in destructive numbers the next spring. The fall of 1906 was unusually dry and warm, the mild weather continuing until late. The larvæ continued feeding until December 6.

On November 4 and 5, 1915, an assistant was able to find larvæ in nearly every field of grain examined in Fergus County. They were not very abundant, but were easily found by the holes eaten in the leaves. At this time the worms were very small, probably in the second instar. Cold weather occurred soon afterward and larval feeding must have ceased. On April 10, 1916, some of the same fields were visited again and the larvæ were still very small. They certainly were very much smaller than on the same date in 1915. It is clear that there is a considerable variation in the size reached before winter sets in and, hence, in the size of the larvæ in the spring.

## HIBERNATION OF THE INSECT

From the foregoing and from Johnson's observations[1] it is clearly evident that the insect hibernates as a partly grown larva.

It has been stated above that in the fall of 1906 the larvæ fed until December 6. The feeding of the caterpillars ceased with the coming of a snowstorm. A field which had been visited only a few days before and which was known to contain many larvæ was examined after this storm. The snow was swept away with a broom, and the larvæ were found on

[1] Johnson, S. A. Cutworms. *In* Colo. Agr. Exp. Sta. Bul. 98, p. 18. 1905.

and near the surface of the soil in a torpid condition. When taken into the hand, they immediately warmed up and began to crawl. There was apparently an absence of any quiescence other than torpor induced by cold.

## DURATION OF LARVAL FEEDING IN THE SPRING

In Montana the larvæ resume activity with the beginning of the growth of vegetation, which is generally in the latter part of March or early in April. In 1910 the first larvæ from the field were sent in on April 24. In 1915 the first to be received at the Experiment Station came on April 2. By April 15 the station was receiving many urgent requests for information regarding control, indicating that the worms were very active. They continued in abundance in the field until about the third week in April and gradually disappeared until early in May, as indicated by many observations in the field and by the correspondence. In 1915 several lots of larvæ from various parts of the State were reared in the insectary and began pupating on April 22 and continued until May 19, when the last had transformed. The greater part of these had pupated by May 10.

In general, the occurrence of the larvæ in "armies" may be said to extend from April 1 to May 1.

## PUPATION AND EMERGENCE OF ADULTS

From the notes of the writer on the rearing and more especially from information regarding the disappearance of the larvæ in the field it is clearly evident that by the last week in April in average years the larvæ are rapidly disappearing. Pupation takes place in an earthen cell about 2 inches under the surface of the soil. The pupa always rests with the anterior end uppermost, and the molted skin lies beside it.

The duration of the pupal stage, as indicated by the rearing records of many isolated individuals in the insectary and not including rearings conducted during the winter months in an artificially heated greenhouse, varies from 43 to 63 days and averages 54.7 days. From field observations the duration of this stage has been determined to be approximately 60 days. The first week in May clearly marks the height of pupation out of doors. On July 16 and 17 fresh moths were found in great abundance in the fields at Willow Creek, while only a very few pupæ could be found. It may be safely assumed that the last of the moths were emerging about this date, and the height of emergence was during the first week in July.

The writer several times has noted that a small advantage of temperature markedly hastens the appearance of the moths of this insect. If kept in a cool place, the emergence of the moths may be greatly delayed. That the time of emergence varies in different seasons is shown by the fact that on July 8, 1910, in attempting to get pupæ 8 miles west of Bozeman it was found that the moths had all emerged. This was an early, dry season. It is quite clear that the first of the moths appear in June.

## NUMBER OF BROODS

From the foregoing observations it is clearly evident that the army cutworm passes through but one annual life cycle. There is not time enough for a second brood to occur between the appearance of adults in the early part of July and the laying of eggs about October 1. Nine days is the sortest incubation period the writer has noted. The only accurate data of the writer regarding the duration of the larval stage were obtained by rearing to maturity in a heated greenhouse in the winter of 1915–16 the various lots of eggs laid by moths in October, 1915. It is believed that the larval period in the greenhouse was probably shorter than it would have been out of doors even in the summer time. The longest period recorded was 118 days, the shortest 96, and the average was 104.06 days. As stated above, the shortest pupal period secured in the indoor rearings was 43 days, which added to the minimum periods gives 148 days, while from July 1 to October 1 there are only 92 days, thus leaving a difference of 56 days. Moreover, no larvæ have been found at any time during the summer which might belong to a second brood.

Observations given in previous paragraphs which have a bearing on this question may be recapitulated. Moths caught in June and July are bright and fresh; those caught in the fall are rubbed and faded. The ovaries are immature in July, while in September and October they are mature. Moths placed in cages at Bozeman in July and given honey water and clover blossoms daily lived until late in September.

If it be assumed that the brood of moths emerging in June and July live over until fall, meantime growing ova, then the 12 months of the year are all accounted for in one life cycle of the insect.

## SUMMARY

From the foregoing observations the life history of the army cutworm (*Chorizagrotis auxiliaris*) may be summarized as follows:

(1) Egg laying was observed from September 30 to October 12, but possibly occurred for some weeks previous to September 30.

(2) The moths deposit the eggs directly upon the bare soil.

(3) The incubation period is about nine days indoors, but hatching may be delayed by lack of sufficient moisture.

(4) The larvæ feed for a variable period in the fall which terminates with the onset of winter.

(5) The insect hibernates as a partly grown larva.

(6) Activity is resumed by the larvæ with the beginning of plant growth in the spring.

(7) The larvæ feed until about the first week in April, when they enter the earth for pupation.

(8) The moths emerge from the latter part of June to the middle of July.

(9) The moths live over until fall, growing ova on food obtained as adults.

(10.) The army cutworm is single brooded in Montana.

# APHIDOLETES MERIDIONALIS, AN IMPORTANT DIPTEROUS ENEMY OF APHIDS

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## INTRODUCTION

The economic importance of *Aphidoletes meridionalis* Felt was established when the larvæ of this cecidomyiid fly were first observed by the writer at La Fayette, Ind., on June 29, 1912, destroying large colonies of *Aphis setariae* and *Hyalopterus pruni* on plum (*Prunus* spp.). Subsequent observations in the States of Iowa, Wisconsin, Illinois, Michigan, and Indiana emphasize its value as an efficient agency in the natural control of Aphididae.

While most of the data reported in this paper were obtained during July and August, 1912, it was impossible to obtain a specific determination of the species from specimens used in the experiments at that time, but during the past season (1915) Dr. E. P. Felt has kindly determined it as *Aphidoletes meridionalis* from living adults reared from larvæ collected in the same locality and attacking the same kinds of aphids as those used in the experiments of 1912. *Aphidoletes meridionalis* was described by Dr. Felt in 1908 from adults reared from larvæ predacious on the tulip-tree aphis (*Macrosiphum liriodendri* Monl.).[1]

## ECONOMIC IMPORTANCE, NATURAL CHECKS, AND APHIDS ATTACKED

The fact that each larva may destroy dozens of aphids and that these flies are remarkably prolific makes this predator very important and valuable. Many instances were observed where aphid colonies were apparently completely destroyed. For example, on June 6, 1915, the undersides of leaves of catnip (*Nepeta cataria* L.) in the writer's yard were completely covered with *Aphis gossypii*, and at that time *Aphidoletes meridionalis* was just making its appearance in numbers, the eggs and larvæ up to half or possibly two-thirds grown being abundant. A week later (June 13) very few aphids remained, and most of the predacious larvæ had made cocoons on the undersides of leaves between the leaf veins or on the ground at the base of plants, and a few days later only very rarely could a live aphid be found. A few syrphid larvæ, an occasional coccinellid larva or adult, and some aphidiine parasites were present, but the control of the aphis was apparently due entirely to *Aphidoletes meridionalis*.

[1] Felt, E. P. Studies in Cecidomyiidae II. In 23d Rpt. State Ent. N. Y., 1907, p. 384, 397. 1908. (N. Y. State Mus. Bul. 124).

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While most of the data reported in this paper were obtained during July and August, 1912, it was impossible to obtain a specific determination of the species from specimens used in the experiments at that time, but during the past season (1915) Dr. E. P. Felt has kindly determined it as *Aphidoletes meridionalis* from living adults reared from larvæ collected in the same locality and attacking the same kinds of aphids as those used in the experiments of 1912. *Aphidoletes meridionalis* was described by Dr. Felt in 1908 from adults reared from larvæ predacious on the tulip-tree aphis (*Macrosiphum liriodendri* Monl.).[1]

## ECONOMIC IMPORTANCE, NATURAL CHECKS, AND APHIDS ATTACKED

The fact that each larva may destroy dozens of aphids and that these flies are remarkably prolific makes this predator very important and valuable. Many instances were observed where aphid colonies were apparently completely destroyed. For example, on June 6, 1915, the undersides of leaves of catnip (*Nepeta cataria* L.) in the writer's yard were completely covered with *Aphis gossypii*, and at that time *Aphidoletes meridionalis* was just making its appearance in numbers, the eggs and larvæ up to half or possibly two-thirds grown being abundant. A week later (June 13) very few aphids remained, and most of the predacious larvæ had made cocoons on the undersides of leaves between the leaf veins or on the ground at the base of plants, and a few days later only very rarely could a live aphid be found. A few syrphid larvæ, an occasional coccinellid larva or adult, and some aphidiine parasites were present, but the control of the aphis was apparently due entirely to *Aphidoletes meridionalis*.

[1] Felt, E. P. Studies in Cecidomyiidae II. In 23d Rpt. State Ent. N. Y., 1907, p. 384, 397. 1908. (N. Y. State Mus. Bul. 124).

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Although the fly is prolific and constitutes an effective check to the increase of aphids under favorable conditions, the adults are very frail and easily destroyed by unfavorable weather conditions, such as beating rains. They do not, as a rule, make their appearance in appreciable numbers until the latter part of May and probably can not, therefore, be considered as being so generally reliable as a natural means of control as are the hymenopterous enemies belonging to the subfamily Aphidiinae.

This cecidomyiid is a general feeder, attacking almost any species of aphid available, but more often feeding on those which live gregariously upon their hosts. The writer's records show that it attacks the following species: *Aphis asclepiadis* Fitch., *A. avenae* Fab., *A. cardui* L., *A. gossypii* Glov., *A. helianthi* Monl., *A. maidis* Fitch *A. setariae* Thos., *Chaitophorus negundinis* Thos., *Hyalopterous pruni* Fab., *Macrosiphum granarium* Kibby, *M. pisi*, Kalt. *M. sonchella* Monl., *Myzus persicae* Sulz., *Phorodon humuli* Schr., *Rhopalosiphum sonchi* Oestl., *Siphi flava* Forbes, *Siphocoryne pastinacae* L., and *Toxoptera graminum* Rond.

FIG. 1.—*Aphidoletes meridionalis:* Eggs in situ on leaf of rape; *a*, egg, greatly enlarged.

## HISTORICAL SUMMARY

Aside from systematic discussions, very little has been written about *Aphidoletes meridionalis*. There can be no doubt that the larvæ predacious on *Macrosiphum pisi* and referred to by Fletcher in his report for 1900 [1] as a species of Diplosis were *Aphidoletes meridionalis*, and this seems to be the first authentic record in economic literature. A short account of the habits of probably the same species as the one under discussion is given by Webster and Phillips,[2] who refer to it as an enemy of *Myzus persicae* and of the spring grain aphis or "green bug" (*Toxoptera graminum*) and predict that it may possibly become an important factor in the control of *T. graminum*. The writer [3] has referred to this species as an active enemy of the pea aphis (*Macrosiphum pisi*) and other writers have barely referred to it as predacious on aphids.

[1] Fletcher, J. T. Report of the entomologist and botanist. 1900. *In* Canada Exp. Farms Rpts. 1900, p. 212. 1901.

[2] Webster, F. M., and Phillips, W. J. The spring grain-aphis or "green bug." U. S. Dept. Agr. Bur. Ent. Bul. 110, p. 133. 1912.

[3] Davis, J. J. The pea aphis with relation to forage crops. U. S. Dept. Agr. Bul. 276, p. 54. 1915.

## LIFE HISTORY AND DESCRIPTIVE NOTES

The eggs (fig. 1) are very small, elliptical oval, chrome orange in color, paler at the extremities, and measure 0.104 mm. in width and 0.313 mm. in length. They are laid in clusters of from 1 to 12 on foliage amongst a colony of aphids or may be deposited on the dorsum of the aphid itself, as many as 7 having been noted on a single aphid. The number of eggs laid by individual females was determined in two cases (Table I), and it will be noticed that these females laid 116 and 125 eggs each, respectively. The cages used for obtaining eggs were of the ordinary "chimney" type and the results certainly were not above normal and more likely were below normal. The exact length of the egg stage was not accurately determined, but from the approximate records given in Tables I and II and from some more exact miscellaneous records the length of the egg period averages about three days.

TABLE I.—*Records of eggs of two individual females of Aphidoletes meridionalis; La Fayette, Ind., August, 1912*

| Cage No. | Date male and female were introduced into cage. | Date first lot of eggs were counted. | Number. | Hatching and rearing records. | Date second lot of eggs were counted. | Number. | Hatching and rearing records. | Date third lot of eggs were counted. | Number. | Hatching and rearing records. |
|---|---|---|---|---|---|---|---|---|---|---|
| 7654(4) | 1912. Aug. 1 | Aug. 3 | 55 | Eggs hatching Aug. 6; adults Aug. 22–23. | Aug. 5 | 30 | Eggs hatching Aug. 8; adults Aug. 22–24. | Aug. 7 | 11 | Adults Aug. 25–26. |
| 7654(5) | [a]Aug. 2 | Aug. 5 | 17 | Eggs hatching Aug. 8; adults Aug. 24. | Aug. 7 | 22 | Adults Aug. 23–24. | Aug. 9 | 38 | Adults Aug. 25. |

| Cage No. | Date male and female were introduced into cage. | Date fourth lot of eggs were counted. | Number. | Hatching and rearing records. | Date fifth lot of eggs were counted. | Number. | Hatching and rearing records. | Date of death of Aphidoletes female. | Total number of eggs. |
|---|---|---|---|---|---|---|---|---|---|
| 7654(4) | 1912. Aug. 1..... | Aug. 11.... | 20 | Adults Aug. 26–27. | ..... | ..... | ..... | Aug. 11?... | 116 |
| 7654(5) | [a]Aug. 2..... | Aug. 12.... | 35 | ..... | Aug. 15.... | 11 | ..... | Aug. 15.... | 123 |

[a] This pair was observed in copula at 7.30 p. m. on Aug. 2.

Immediately upon hatching the larva attacks the most convenient aphid, and at this stage of its life more often pierces the body of its host from beneath, usually between the legs. After sucking the body fluids from the first aphid and killing it, the larva leisurely moves to another, this operation being continued until it becomes full grown. The larva always seems to move about cautiously, at the same time quickly thrusting its tongue-like anterior end in and out and to all sides much as does a syrphid larva. When it locates its host it thrusts its proboscis into the

aphid and sucks the body fluids until the aphid is dead and more or less shriveled. The victim seldom notices the presence of the larva, judging from outward indications. After the larva becomes one-third grown it usually punctures the aphid at one of the articulations of the legs, a favorite point of attack being at the membranous joint connecting the tibia and femur (Pl. CX, fig. 2). If the larva attacks the aphid at an articulation as above described, the latter seldom notices the attack; but if the proboscis of the larva touches the wrong places, the aphid kicks about more or less for a few seconds. As a rule, several minutes are required for the larva to pump out most of the body juices of the host, but this time varies, depending upon the relative sizes of the larva and its host. The aphid is often discarded by the larva soon after it has been killed and long before it has been sucked dry.

FIG. 2.—*Aphidoletes meridionalis:* Larva, dorsal view. Greatly enlarged.

To the naked eye the larva of *Aphidoletes meridionalis* (fig. 2) closely resembles such common cecidomyiids as the cloverleaf midge (*Dasyneura trifolii* Loew), but differs slightly in coloration, being usually of a pale orange, varying from pale pinkish to a rather deep orange, and when mature measures approximately 3 mm. in length. The length of the larval period varies, depending upon the temperature and food supply; but according to observations of the writer it is between 7 and 11 days.

When fully mature the larva spins a loose cocoon of silk mingled with aphid remains, attaching it to the leaf between the veins; or it descends to the ground and at or near the surface spins its cocoon (fig. 3), incorporating with it particles of dirt and trash. The larva pupates shortly after constructing the cocoon. The pupa (fig 4), which is of an orange color, resembles other related cecidomyiids; it measures 2 mm. in length and its cocoon is 2.25 mm. long and 1.125 mm. wide. The length of the pupal stage varies, according to observations, between 6 and 9 days.

FIG. 3.—*Aphidoletes meridionalis: a,* Cocoon formed on surface of ground; *b,* cocoon formed on a leaf blade.

The adult (Pl. CX, fig. 1) may be popularly described as a small, frail midge, much resembling, to the casual observer, (*Dasyneura*) *Neocerata rhodophaga* Coq. the destructive rose midge, or the clover-seed midge (*Dasyneura leguminicola* Lintn.). Its length is approximately 1.4 mm. for the male, and 1.8 mm. for the female; the body is pale and the abdomen has a decided pinkish tint. Copulation and egg laying seem to occur

at night—at least they have been observed by the writer only at night, although the cages were examined much more frequently during the day.

Egg laying continued over a period of about 10 days in "chimney" cages, and the length of life of the midge under the same conditions was about 14 days. Several unsuccessful attempts were made to induce unfertilized females to lay eggs, although fertilized females laid eggs readily, indicating that this species is probably not parthenogenetic.

FIG. 4.—*Aphidoletes meridionalis:* Pupa, lateral view. Much enlarged.

As will be seen from Table II, the total length of the life cycle from egg to adult varied, in the region in which it was studied, from 15 to 29 days, the average normal life cycle being about 18 to 20 days. The seasonal number of complete generations has not been determined, but there are evidently at least six complete generations annually, the winter being passed as larvæ and possibly also as pupæ within the cocoons.

TABLE II.—*Length of life cycle of Aphidoletes meridionalis; La Fayette, Ind., July-August, 1912*

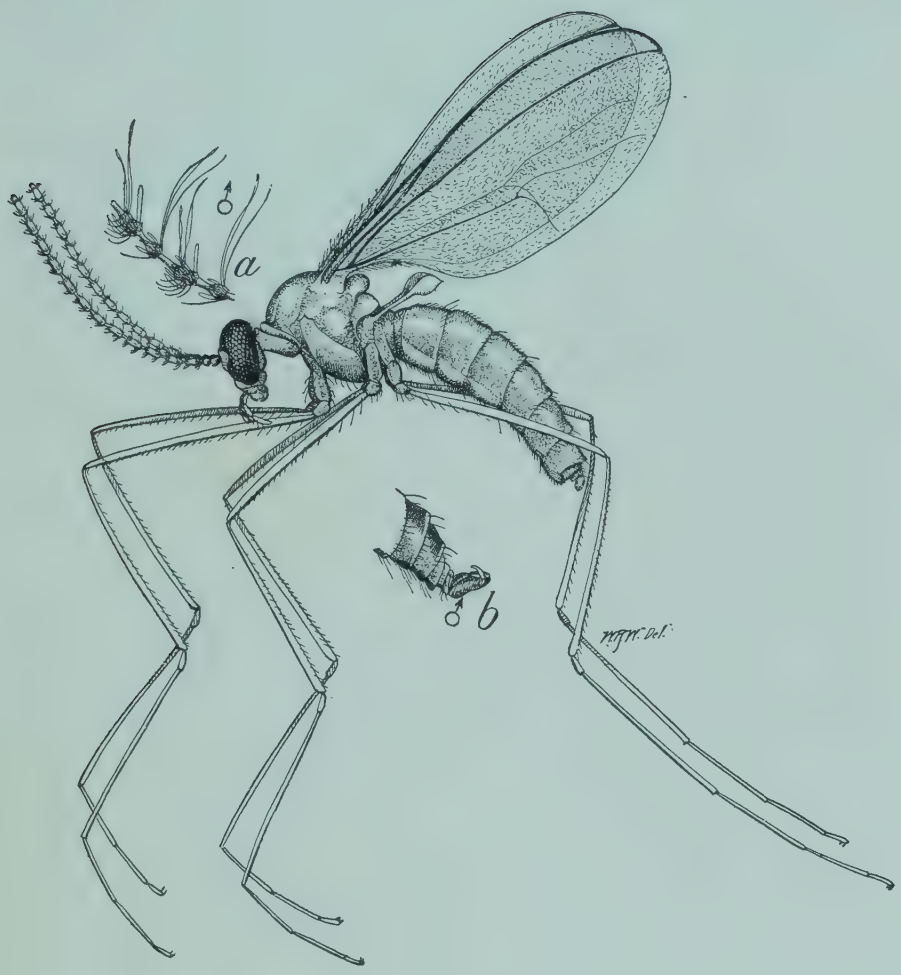
| Cage No. | Eggs laid— | Eggs hatched— | All larvæ finished feeding— | Cocoons noticed— | Adults issued— | Total life cycle. | |
|---|---|---|---|---|---|---|---|
| | | | | | | Minimum. | Maximum. |
| | 1912. | | | | | *Days.* | *Days.* |
| 7654(1)c | July 15–16 | July 17–18 | | | Aug. 5–14 | 20 | 30 |
| 7654(1)d | July 17–18 | July 20 | | July 25–29 | Aug. 5–13 | 18 | 27 |
| 7654(3)a | Aug. 1 | Aug. 5–6 | Aug. 13 | | Aug. 21 | 20 | |
| 7654(4) | Aug. 9–11 | | | | Aug. 26–27 | 15 | 18 |
| 7654(4)a | Aug. 1–3 | Aug. 6 | Aug. 15 | | Aug. 22–23 | 19 | 22 |
| 7654(4)b | Aug. 3–5 | Aug. 7–8 | ...do | | Aug. 22–24 | 17 | 21 |
| 7654(4)c | Aug. 5–7 | | Aug. 14 | | Aug. 25–26 | 18 | 21 |
| 7654(5)a | Aug. 3–7 | Aug. 7–8 | | | Aug. 24 | 19 | 21 |
| 7654(5)b | Aug. 5–7 | | | | Aug. 23–24 | 16 | 19 |
| 7654(5)c | Aug. 7–9 | | | | Aug. 25 | 16 | 18 |
| 7654(2)a | July 15–16 | July 18 | July 25 | July 24–26 | Aug. 1–13 | 16 | 29 |
| 7654(2)b | July 16–17 | | | July 26 | Aug. 4–7 | 18 | 22 |

## PLATE CX

*Aphidoletes meridionalis:*

FIG. 1.—Adult female: *a*, Antenna of male, showing structure; *b*, tip of male abdomen. Greatly enlarged. (Redrawn after Webster and Phillips.)

Fig. 2.—Larva attacking a pea aphis (*Macrosiphum pisi*). (From Davis.)

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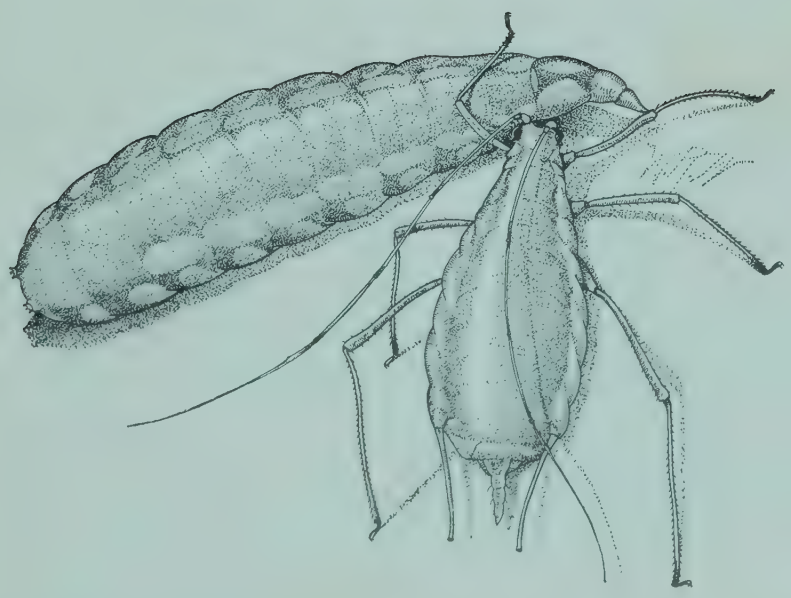

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# INFLUENCE OF BARNYARD MANURE AND WATER UPON THE BACTERIAL ACTIVITIES OF THE SOIL

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## INTRODUCTION

The application of barnyard manure to a soil brings about a far-reaching change within the soil. It has been found that, as an average, 1 ton of barnyard manure contains 10 or 12 pounds each of nitrogen and potassium and 2 or 3 pounds of phosphorus. It also carries other substances of less importance which may be directly utilized by the growing plant or which may react with substances within the soil, changing their solubility. This direct and indirect nutritive value of a manure is not its only function, for it changes greatly the physical structure of the soil. It improves the tilth of a clay soil by increasing the granulation within it, while in a sandy soil it tends to bind the particles together, making it less porous. Each of these changes react upon the water-holding capacity and the capillarity of the soil, greatly altering the aeration of the soil and with it the temperature.

The biological changes which the manure produces in the soil, especially when small quantities are added, may be more far-reaching than either the chemical or physical changes which it produces. Every pound of manure carries with it to the soil millions of bacteria. Many of these will find the new conditions unsuited for their growth, but some will continue to multiply, and in so doing not only will decompose the constituents of the manure but also will greatly alter other organic and inorganic substances of the soil. Hence, the bacterial content of the soil is changed both quantitatively and qualitatively. There are added with the manure many new species, and the changed physical and chemical conditions of the soil due to the manure will greatly modify those already present, for the microflora and fauna originally present in the soil were due to specific properties of the soil.

This changed flora and fauna will in turn change the chemical and physical properties of the soil still more. Acids are generated, which react with insoluble constituents, rendering them soluble. Gases are formed, which change the air within the soil; and in these reactions heat is generated, thus changing the temperature of the soil. The metabolism of the bacterial cell requires nutritive substances, among which are water

[1] The authors wish to express their appreciation of the kindness of Dr. F. S. Harris, of the Utah Experiment Station, in placing at their disposal the plots used in this investigation and also the records of treatment and yield, for it is this assistance which has made possible this investigation.

and the elements essential to plant growth. Some soluble constituents will be changed to insoluble and some inorganic to organic. All of these changes must be reflected in the yield of the crop produced.

This investigation was undertaken to throw more light on some of these changes, especially the influence of manure in the presence of varying quantities of water upon the bacterial activities of the soil, and it may be seen by an examination of the more important literature on the subject that with respect to the control of both manure and moisture this experiment is unique.

## HISTORICAL REVIEW

That the addition of manure to a soil increases the number of bacteria has been shown by Remy[1] (37, p. 660–733) and Fischer (13, p. 358). Caron (6) found that the number of bacteria present depends not only upon the manure added but upon the cultural methods and the crop grown upon the soil. Fabricius and Von Feilitzen (12) found that bacteria increased in the soil on the addition of manure and that a direct relationship existed between the temperature of a soil and the number of bacteria found in it. That the temperature of the soil is influenced by the addition of manure is shown by Wagner (47), who observed that manure increased the temperature of soil from 1 to 2.8 degrees centigrade, depending on the kind and condition of manure added. Troop (44) noted an average increase of 5 degrees in temperature of soil receiving 25 tons per acre of manure over unmanured soil. Petit (35), however, claimed that, while there was at first an increase in the temperature of manured soils, later it became lower than the unmanured. Stigell (41) concluded that bacteria under favorable conditions for development retarded the conduction of heat in soils and thereby reduced the temperature changes due to the variation in the outside temperature. This in a way might neutralize the effect of manure, for Hecker (20) found that while the temperature of soil to which well-rotted manure had been added was higher than adjacent unmanured soil during the day, the opposite was true during the night. Grazia (17) stated that manures greatly increase the temperature of the soil. King (26) found that a definite increase in bacterial activity occurred with increased temperature, but that an excessive moisture content greatly reduced the number of bacteria in a soil. Engberding (11) claimed that manure increased the number of bacteria in a soil, but he considered that the moisture content had a greater influence on numbers than did temperature. That the moisture content greatly influenced bacterial activity was shown by Dehérain and Demoussy (9), who found that the bacterial action of a soil was at its maximum when a rich soil contained 17 per cent of water, but that it decreased if the proportion of water fell to 10 per cent or rose to 25 per

[1] Reference is made by number to "Literature cited," p. 923–926.

cent. With soils less rich in humus a somewhat higher proportion of water was necessary to retard oxidation to any marked degree. In a manured soil the coarse manure tended to cause the surface soil to dry out, while fine manure prevented evaporation. King (25) observed that manured land contained more moisture throughout the year than unmanured soil, and this was reflected upon both the bacteria and the crop. The bacteria themselves may play a small part in this difference in moisture content, as was shown by Stigell (42), who found that bacteria decreased the speed of evaporation of water from Petri dishes. Hiltner and Störmer (24) claimed that the addition of manure to a soil brought about a marked increase in the number of bacteria. The temperature, cultural methods, and crop had an influence, but it was not nearly so pronounced as that produced by the manure. Dafert and Bolliger (8) stated that the difference in moisture did not have to be great to produce a great change in the oxidation going on in the soil, for a distinctly measurable difference was noted when the moisture varied 1 per cent.

Brown (4), in a study of the influence of manure on the bacterial activities of a loam soil, found that applications of manure up to 16 tons per acre increased the number of bacteria and also the ammonifying and nitrifying powers of the soil. The greatest increase in the processes was brought about by small applications of manure, 8 to 12 tons to the acre. He observed a close relationship between the ammonifying powers of the soil, the bacterial content, and the crop produced on the soil.

Temple (44) stated that the addition to a soil of 10 tons of cow manure per acre greatly increased the number of bacteria in the soil, but that a greater increase occurred when a sterilized manure was applied. This, however, is not in keeping with the results obtained by other investigators, for Hellström (22) concluded that manures possessed a fertilizing effect aside from the quantities of fertilizer constituents contained within them; and this, he claimed, is their great bacterial content. And Stoklasa (43) found that manure increased the bacterial content and activity of a soil and was greater with small, frequent applications of manure than with large applications made at longer intervals. Moreover, Lipman and others (31) observed that the bacteria conveyed to soil in small quantities of manure were valuable in bringing about a more rapid decomposition of a green-manure crop, while Briscoe (3) said that a direct relationship existed between the organic matter added to a soil and the bacterial count and that a light dressing of manure with green manure produced a marked effect upon both the crop and the bacterial count. Bacterial cultures added with the green manure gave just as pronounced an effect as did the stable manure. Lemmermann and Einecke (29), however, obtained no increase on adding stable manure with green manure. This may be due to the different kind of manure used, for Emmerich and others (10) claimed that a more favorable effect

was obtained from the use of well-rotted manure than fresh manure. This, they claimed, was due to the production in the latter of formic, acetic, and butyric acids, indol, skatol, and hydrogen sulphid, which are toxic to the plant. Under some conditions the large quantities of carbon dioxid liberated from the rapidly decomposing fresh manure may be valuable in rendering soluble plant food. Bornemann (2) found that soil constantly supplied with carbon dioxid through a pipe buried in the ground gave an increase in yield of 12.2 per cent over the crop grown on untreated soil. Wollny (52) has shown that manure greatly increased the carbon-dioxid production in a soil.

Moll (33) claimed that the season of the year and not the kind of fertilizer used, nor even the weather conditions, is the principal factor in determining the peptone decomposition, nitrification, and nitrogen fixation of a soil. According to Wohltmann, Fischer, and Schneider (51), ammonification, nitrification, and nitrogen fixation were all more or less increased by the application of manure. Lipman (30, p. 135) found that the peptone-decomposing power of a soil was greatly increased by the application of manure. Heinze (21) found that manure was especially beneficial to the nitrifying organisms. Warington (48) reports that much more nitric nitrogen was found in the soil of plots which had received annually for 38 years a dressing of 14 tons of manure to the acre than in any of the other manured or unmanured plats. While Stevens (39) found that nitrification was much more active in manured than in unmanured soil, Frankfurt and Duschechkin (14) observed an increase in nitrification only on those manured plots on which the yield had increased. Velbel (46) has shown that the chief factors controlling nitrification in fallow soil were the humus and the humus-nitrogen content of the same, the nitrification having increased directly with the humus. He noted, however, a certain amount of denitrification at first, but later in the summer nitrification became more rapid on the manured than on the unmanured soil, the effect of the manure being still perceptible after four years. Some investigators (23, 36, 50) have reported a reduction of nitrates, but the quantity of manure applied was excessive, or else of a very coarse nature, or the soil very poorly aerated. Barthel (1) found that nitrification did not take place in the presence of soluble organic matter, but he considered it unlikely that sufficient quantities of soluble organic constitutents occurred in normal agricultural soils to interfere greatly with nitrification. Niklewski (34) claimed that nitrification occurred in solid stable manure when there was not much liquid present. He stated that on the first day some nitrite bacteria were present and at the end of four weeks there were 10,000 per gram. Associated with these were nitrate bacteria which were identical with those isolated by Winogradsky. Millard (32), however, was unable to find many nitrifying bacteria in manure.

Many of the cases in which individuals have reported a disappearance of nitrates in soil are due to synthetic reactions, the nitrates being built up into complex proteins. For Gerlack and Vogel (15) have shown that there are several varieties of bacteria in the soil which have the power of converting ammonia, nitrites, and nitrates into insoluble proteins.

The processes of ammonification, nitrification, and nitrogen fixation, being due to the action of micro-organisms, are intimately associated with the moisture content of the soil; hence, we find in many cases this is the limiting factor. Guistiniani (16) found in sandy soil that the rapidity of nitrification of ammonium sulphate was directly proportional to the amount of moisture present when this varied from 0 to 16 per cent. While Roche (38) has shown that irrigation supplying from 15 to 25 per cent of water to a soil furnished the most favorable conditions for nitrification, Coleman (7) found nitrification most active in a loam soil with a moisture content of 16 per cent. It was greatly retarded when the water content was reduced to 10 per cent or raised to 26 per cent. It is also interesting to note that he found that with a high moisture content soluble organic matter became injurious to nitrification.

The nitrogen-fixing organisms would also be influenced by the water content, as shown by Warmbold (49), who stated that when the water content went below 10 per cent there was no nitrogen fixation and in some cases there was a decided loss of nitrogen. Krainskiĭ (27) said that nitrogen fixation was at its height in soils containing fairly small quantities of water. Later he (28) stated that the higher the humus content the larger the water content of the soil required for optimum nitrogen fixation. Increasing the organic matter of the soil was not found to increase nitrogen fixation, although there was an increased bacterial activity. Hanzawa (18) found that the humus of stable manure could be used as a source of energy by some nitrogen-fixing bacteria.

## PLAN OF EXPERIMENT

The plan of the experiment is such that it can be divided into three parts. The first deals with the bacterial activities of a soil receiving a definite amount of manure and measured quantities of irrigation water and kept fallow in pots under vegetation house conditions. In this the moisture content could be accurately maintained by the weekly weighing and the replacing of lost moisture. The variation in temperature and moisture of this series would not be as great as it would be under field conditions. The second part deals with the bacterial activities going on in a soil under field conditions, the soil receiving known quantities of manure and water but kept fallow. The third part deals with soil of the same field under irrigated conditions and manurial treatment the same as the second part, but producing a crop.

## COMPOSITION OF SOIL

The investigation was conducted either on soil from the Greenville Experiment Farm or on the farm itself, which is situated 2 miles north of Utah Agricultural College. The soil represents a type found in large areas in the Great Salt Lake Basin. It is of a sedimentary nature, being derived from the weathering of the mountain range near by, which consists largely of limestone and quartzite deposited by the streams as they flowed into the now extinct Lake Bonneville. The soil is situated at the foot of the main delta thus formed and consists of fine sand and coarse silt of fairly uniform chemical and physical composition to a great depth. The chemical and physical analysis of the soil is given in Table I. The chemical analysis was made according to the official methods of the Association of Official Agricultural Chemists,[1] while the physical analysis was made by means of the Yoder soil elutriator.

TABLE I.—*Chemical and physical composition of the soil of the Greenville (Utah) Experiment Farm*

| Chemical composition. | | Physical composition. | |
|---|---|---|---|
| Constituent. | Per cent. | Constituent. | Per cent. |
| Insoluble residue | 41.46 | Coarse sand | 0.21 |
| Soluble silica | .62 | Medium sand | 9.63 |
| Total | 42.08 | Fine sand | 30.04 |
| Potash ($K_2O$) | .67 | Coarse silt | 32.25 |
| Soda ($Na_2O$) | .35 | Medium silt | 12.30 |
| Lime (CaO) | 16.88 | Fine silt | 6.25 |
| Magnesia (MgO) | 6.10 | Clay | 7.62 |
| Oxid of iron ($Fe_2O_3$) | 3.03 | Moisture | 1.60 |
| Alumina ($Al_2O_3$) | 5.64 | Soluble and lost | .10 |
| Phosphoric acid ($P_2O_5$) | .41 | Specific gravity | 2.67 |
| Carbon dioxid ($CO_2$) | 19.83 | Apparent specific gravity | 1.23 |
| Volatile matter | 5.60 | Water-soluble salts | .06 |
| Total | 100.69 | | |
| Humus | .53 | | |
| Nitrogen | .139 | | |

The soil has been analyzed to a depth of 10 feet and was found to be very similar in both chemical and physical composition to that given in Table I. There were, however, slightly greater quantities of acid-soluble material in the lower foot sections. The humus and nitrogen of the deeper soil was slightly less than in the first foot. The physical composition is practically the same to a depth of 10 feet. The soil is exceptionally rich in phosphorus and potassium, but low in nitrogen and humus. The calcium and magnesium contents are exceptionally high and one may conclude that for this reason the soil is unproductive; but just the reverse is true, for the soil is very fertile and even with its low nitrogen and humus content produces excellent crops.

[1] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted 1912.

## METHOD OF SAMPLING THE SOIL

All possible precautions against the contamination of one sample by another were taken in collecting them. The surface soil to a depth of half an inch was scraped off by means of a sterile spade. A hole 12 inches deep was dug, and a slice of soil to this depth was taken from the side of the hole and placed in a sterile mixing pan. This process was repeated from four or five places in the field and then the contents of the pan carefully mixed by means of a sterile spatula. From this composite sample a representative portion, about 5 pounds of soil, was placed in a sterile ore sack and conveyed to the laboratory for analysis.

Before each sampling, the spade, mixing pan, and spatula were all carefully sterilized by heat from a plumber's torch, thus preventing the transfer of organisms from one soil to another. The samples were immediately transferred to the laboratory, partly air-dried in the dark, and then ground in a sterile mortar, all coarse rock being removed. The analysis was begun in all cases within 24 hours of the time of taking samples.

## METHODS OF SOIL ANALYSIS

The number of organisms were determined by growing on modified synthetic agar having the following composition:

1,000 c. c. of distilled water.
10 gm. of dextrose.
0.5 gm. of dipotassium phosphate ($K_2HPO_4$).
0.2 gm. of magnesium sulphate ($MgSO_4$).
2 gm. of powdered agar per 100 c. c. of media.

After the samples of soil had been carefully mixed by shaking 100 gm. were weighed on a sterile watch glass, using a small sterile spatula. This soil was transferred to 200 c. c. of sterile water and shaken for one minute, 1 c. c. of this suspension transferred to 99 c. c. of sterile water, and the dilution continued with 9 c. c. of sterile water. The plates were made so as to give a dilution of 1 to 20,000 and 1 to 200,000. They were incubated at 28° C. for four days and then counted. No attempt was made to differentiate between bacteria and molds, but all were listed together as total numbers of colonies.

The ammonifying power of the soil was determined by weighing 100-gm. portions of the soil and 2 gm. of dried blood into sterile tumblers and covering them with Petri dishes. The dried blood was thoroughly mixed with the soil by means of a sterile spatula and the water content made up to 18 per cent with sterile water. The samples were incubated at 28° to 30° C. for four days and the ammonia determined by transferring to Kjeldahl flasks with 250 c. c. of distilled water, adding 2 gm. of magnesium oxid and distilling into *N/10* sulphuric acid. The determinations were all made in duplicates and compared with sterile blanks.

The nitrifying power of the soils was determined in tumblers, like the ammonifying power, except that they were incubated for 21 days. The moisture content was made up weekly to the initial 18 per cent.

At the end of the incubation period each soil was transferred with 250 c. c. of distilled water to a 1-pint Mason fruit jar. Two gm. of powdered lime were added and the jar placed in the shaking machine for 10 minutes, after which it stood in the closed jar until clear. This never required over two hours. At the end of this time an aliquot part, 100 c. c., was measured into a flask and the nitrates determined by the aluminum reduction method (5).

The nitrogen-fixing powers of the soil were made by weighing 5-gm. portions of the soil into 500 c. c. Erlenmeyer flasks containing 100 c. c. of Ashby solution. These, together with sterile blanks, were incubated for 18 days and then the total nitrogen determined by the Kjeldahl method. All determinations were made in triplicate.

## POT EXPERIMENTS

Dry soil, to a depth of 12 inches, was taken from one of the unmanured plots of the Greenville Farm and very carefully mixed and used as the soil for the pot experiments. This soil, together with the required quantity of well-rotted barnyard manure, was packed into the pots. Moisture determinations were made upon the mixtures and then sufficient water added to make up to the required moisture content. The pot and contents were weighed and the moisture content made up weekly to the initial content. The pots were kept on shelves within the building for four months, and then the various determinations were made on each sample as outlined. The temperature of the soil was taken each time before making it up to the moisture content. The manure was applied at the rate of none, 5, 10, 15, 20, and 25 tons to the acre. An acre of soil was considered as weighing 2,000,000 pounds. Each ton of the manure contained 738 pounds of dry matter, 3.04 pounds of phosphorus, 13.70 pounds of potassium, and 16.08 pounds of nitrogen. The moisture was kept at 12.5, 15, 17.5, 20, and 22.5 per cent by weight. Duplicate pots were used in every case with each specific treatment. At the end of the experiment three separate analyses made on each pot, so that each reported result is the average of six closely agreeing determinations. The results are given in Table II.

The number of bacteria developing on synthetic agar does not seem to have been greatly influenced by the various treatments. All counts are comparatively low. If, however, we average the results for all pots which received the same manurial treatment we find a greater number developed from the soils which received 25 tons of manure to the acre than from any of the others. Moreover, there is an appreciable difference in favor of those soils receiving from 10 to 20 tons per acre over the unmanured soil. The irrigation water apparently depresses the number of organisms, for the greatest number developed from soil receiving the least water; but here also the difference is not marked or regular. The

average counts from the pots receiving the same quantities of irrigation water are with 12.5 per cent of water, 4,251,000; 15 per cent of water, 3,838,000; 17.5 per cent of water, 3,882,000; 20 per cent of water, 3,352,000; and 22.5 per cent of water, 3,680,000.

TABLE II.--*Number of bacteria developing on synthetic agar and quantity of ammonia and nitric nitrogen formed in 100 gm. of soil and of nitrogen fixed in 100 c. c. of Ashby solution—pot experiments*

| Treatment. | Number of colonies of bacteria. | Quantity of ammonia formed. | Quantity of nitric nitrogen formed. | Quantity of nitrogen fixed. |
|---|---|---|---|---|
| | | *Mgm.* | *Mgm.* | *Mgm.* |
| 12.5 per cent of water; no manure | 3, 530, 000 | 36. 9 | 3. 36 | 9. 9 |
| 12.5 per cent of water; 5 tons of manure | 3, 300, 000 | 37. 9 | 5. 81 | 10. 3 |
| 12.5 per cent of water; 10 tons of manure | 4, 710, 000 | 48. 5 | 78. 05 | 10. 19 |
| 12.5 per cent of water; 15 tons of manure | 2, 810, 000 | 49. 1 | 88. 55 | 9. 94 |
| 12.5 per cent of water; 20 tons of manure | 6, 100, 000 | 49. 6 | 115. 00 | 10. 15 |
| 12.5 per cent of water; 25 tons of manure | 5, 060, 000 | 60. 5 | 110. 55 | 9. 73 |
| 15 per cent of water; no manure | 3, 360, 000 | 37. 6 | 4. 90 | 10. 19 |
| 15 per cent of water; 5 tons of manure | 3, 300, 000 | 43. 2 | 67. 90 | 10. 33 |
| 15 per cent of water; 10 tons of manure | 3, 660, 000 | 49. 0 | 84. 87 | 11. 50 |
| 15 per cent of water; 15 tons of manure | 4, 260, 000 | 51. 0 | 110. 55 | 11. 90 |
| 15 per cent of water; 20 tons of manure | 3, 720, 000 | 57. 1 | 113. 42 | 11. 02 |
| 15 per cent of water; 25 tons of manure | 4, 730, 000 | 74. 8 | 117. 25 | 10. 05 |
| 17.5 per cent water; no manure | 3, 800, 000 | 37. 4 | 3. 49 | 10. 57 |
| 17.5 per cent of water; 5 tons of manure | 3, 730, 000 | 48. 5 | 75. 60 | 10. 50 |
| 17.5 per cent of water; 10 tons of manure | 4, 330, 000 | 48. 6 | 88. 21 | 11. 46 |
| 17.5 per cent of water; 15 tons of manure | 4, 050, 000 | 54. 7 | 108. 85 | 9. 59 |
| 17.5 per cent of water; 20 tons of manure | 2, 920, 000 | 60. 9 | 111. 80 | 9. 69 |
| 17.5 per cent of water; 25 tons of manure | 4, 460, 000 | 69. 9 | 124. 40 | 9. 83 |
| 20 per cent of water; no manure | 3, 330, 000 | 38. 8 | 4. 20 | 9. 45 |
| 20 per cent of water; 5 tons of manure | 2, 860, 000 | 50. 0 | 73. 32 | 10. 36 |
| 20 per cent of water; 10 tons of manure | 4, 030, 000 | 54. 4 | 78. 14 | 10. 81 |
| 20 per cent of water; 15 tons of manure | 3, 430, 000 | 65. 4 | 106. 55 | 11. 20 |
| 20 per cent of water; 20 tons of manure | 3, 230, 000 | 69. 7 | 113. 00 | 10. 46 |
| 20 per cent of water; 25 tons of manure | 3, 230, 000 | 67. 9 | 125. 70 | 10. 60 |
| 22.5 per cent of water; no manure | 3, 730, 000 | 36. 2 | 7. 85 | 10. 54 |
| 22.5 per cent of water; 5 tons of manure | 4, 400, 000 | 48. 8 | 65. 74 | 10. 57 |
| 22.5 per cent of water; 10 tons of manure | 3, 030, 000 | 60. 8 | 81. 19 | 11. 83 |
| 22.5 per cent of water; 15 tons of manure | 3, 130, 000 | 63. 8 | 118. 50 | 10. 43 |
| 22.5 per cent of water; 20 tons of manure | 4, 530, 000 | 63. 1 | 119. 30 | 10. 95 |
| 22.5 per cent of water; 25 tons of manure | 3, 260, 000 | 64. 6 | 126. 65 | 10. 89 |

Both the water and the manure applied make a marked difference in the ammonifying powers of the soil. It is lowest in those pots which received no manure and gradually increases when 5, 10, 15, 20, and 25 tons of manure are applied. The water likewise has a noticeable effect on the ammonifying powers of the soil. In the unmanured soil it increases until 20 per cent of water is applied, at which point it reaches its maximum. When more than this quantity of water is applied, the ammonification is retarded. It is not as great when 22.5 per cent of water is applied as in the presence of only 12.5 per cent. Similar results are obtained when various quantities of water are applied in the presence of 5 tons of manure per acre. Here the influence of the water is much more pronounced than it is in the absence of manure. It reaches its maximum effect when 20 per cent of water is applied. In the presence of 10 tons per acre of manure the higher percentages of water have much greater influence on the ammonifying powers of the soil than do the lower percentages of water. In the presence of 20 tons of manure the water also exerts a great influence, but here the higher percentages produce a depressing effect, which becomes very perceptible in the pots which have received 25 tons of manure to the acre. It is interesting to note that with 25 tons of manure 15 per cent of water gave better results than either higher or lower percentages of water. It is quite possible that the higher water content in the presence of large quantities of organic matter produce anerobic conditions which are not fully compatible with the best bacterial activities. The results are brought out more fully in figure 1, on the horizontal line of which is given the percentage of water applied to the soil, while on the perpendicular line is given the milligrams of ammonia produced in 100 gm. of soil.

If we consider the average quantity of ammonia produced in the unmanured pots as 100 per cent, that produced on the various manured pots becomes, with 5 tons of manure, 122 per cent; with 10 tons of manure, 140 per cent; with 15 tons, 152 per cent; with 20 tons, 160 per cent; and with 25 tons, 181 per cent. The average increase per ton of manure applied is greatest when 5 tons to the acre are applied and becomes gradually less as the quantity of manure applied becomes greater. If we consider the average percentage of ammonia produced in the soils receiving 12.5 per cent of water as 100, then the soil receiving 15 per cent of water produced 110 per cent; the soils with 17.5 per cent of water produced 111 per cent; the soils with 20 per cent of water, 123 per cent; and those receiving 22.5 per cent of water produced 119 per cent of ammonia—a gradual increase in the ammonia produced until the quantity of water applied exceeded 20 per cent.

The application of manure to a soil produces a very great increase in the nitrifying powers of the soil. The quantity of nitrates produced is very low in the soil receiving no manure but is greatly increased with the application of manure, even with so large a quantity as 25 tons per

acre. There is nothing in the results which would indicate denitrification in the presence of the largest quantities of organic matter applied in this experiment. The nitrifying powers of the soil increase with the water applied up to 17.5 per cent. Above this it has a slight depressing effect upon nitrification, probably caused by the production of an anerobic condition, but even with the highest percentage of water and 25 tons per acre of manure there is nothing in the results obtained which would indicate that denitrification had taken place. These results are brought out clearly in figure 2, in which is indicated on the horizontal line the per-

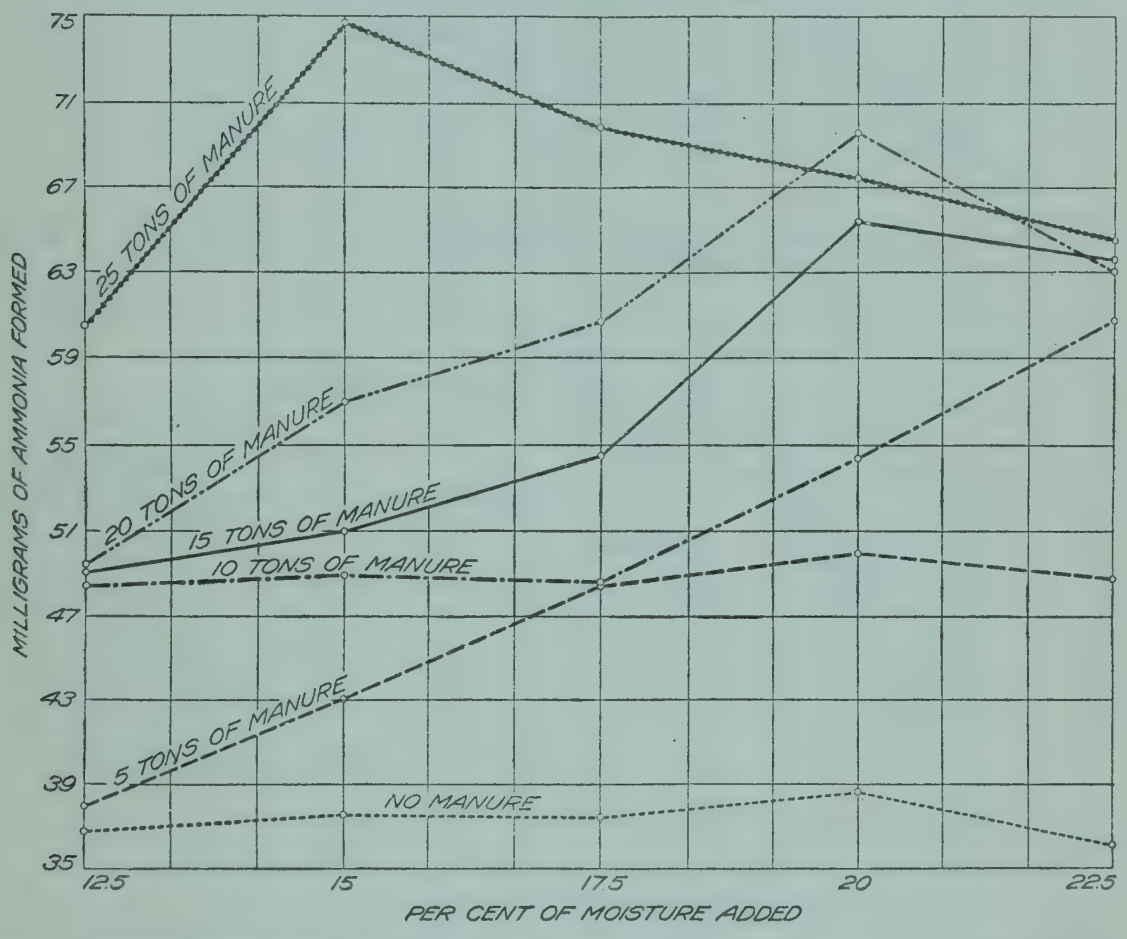


FIG. 1.—Curves of the ammonifying powers of soil in pots with varying quantities of manure and water.

centage of water and on the perpendicular line the milligrams of nitric nitrogen produced in 100 gm. of soil.

If we take the average of the nitric nitrogen produced in the unmanured pots as 100, then that of the manured pots becomes with 5 tons of manure, 1,211 per cent; with 10 tons, 1,762 per cent; 15 tons, 2,240 per cent; 20 tons, 2,405 per cent; and 25 tons, 2,540 per cent. The greatest increase per unit of manure is produced when 5 tons of manure are applied. The water applied also produces a gradual increase, but here likewise the greatest increase per unit of water applied is greatest for the lowest application of water.

The nitrogen-fixing powers of all the soils are fairly high, but the influence of the water and manure is not as pronounced as it is upon the

ammonifying and nitrifying powers of the soil. The results as a whole indicate that the manure increases the nitrogen-fixing power of the soil and it is slightly higher when 10 tons per acre of manure are applied to a soil than when any of the other quantities are applied. Even those

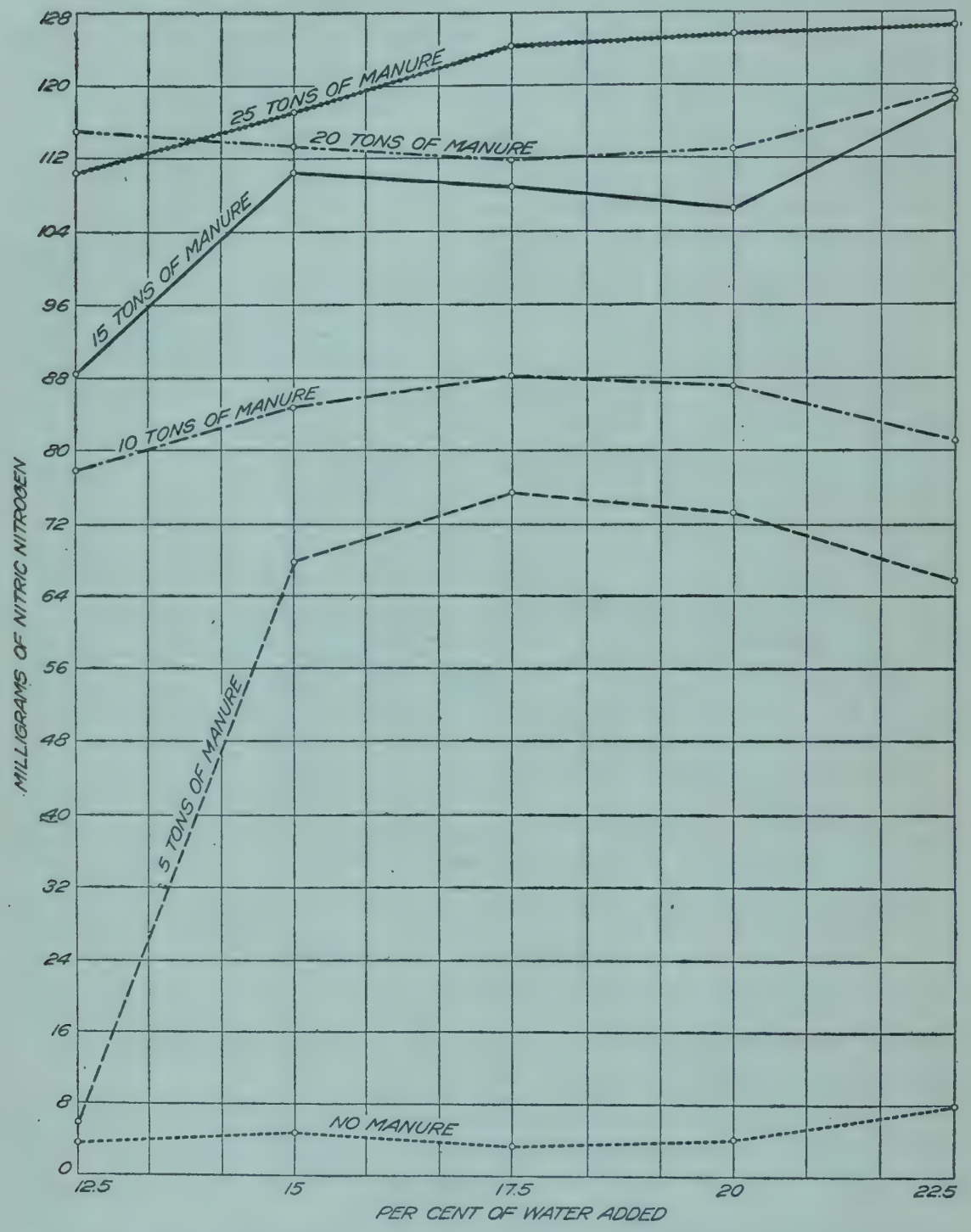


FIG. 2.—Curves of the nitrifying powers of soil in pots with varying quantities of manure and water.

pots receiving 5, 15, and 20 tons of manure per acre as an average fix more nitrogen than the unmanured soil.

The results taken as a whole indicate that the application of manure to soils in pot experiments influenced to a very great extent the ammonifying and nitrifying powers of a soil, but the influence upon the num-

ber of bacteria and nitrogen-fixing powers of the soil, while perceptible, is not as regular. The application of manure produced no difference in the temperature of the soil. The temperature of the manured and unmanured soils averaged very nearly the same throughout the experiment. The temperature of the pots receiving the least quantities of water averaged 1 degree centigrade higher than the soils receiving the greatest quantity of water.

The relationship existing in the various bacterial activities of the soil is brought out best by taking the average of each set of pots receiving the same quantity of manure and water. Then, if the bacterial activities of the pots receiving no manure and that of the pots receiving 12.5 per cent of water each be taken as 100 per cent and the others on a similar basis, we obtain a direct comparative value for each treatment. The results so obtained are given in Table III.

TABLE III.—*Bacterial activities of the soil in the presence of varying quantities of manure and water—pot experiments*

| Treatment. | Bacteria. | Ammonia. | Nitric nitrogen. | Nitrogen fixed. |
|---|---|---|---|---|
| | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| No manure | 100 | 100 | 100 | 100 |
| 5 tons of manure | 99 | 122 | 1,211 | 103 |
| 10 tons of manure | 111 | 140 | 1,762 | 110 |
| 15 tons of manure | 100 | 152 | 2,240 | 105 |
| 20 tons of manure | 116 | 160 | 2,405 | 103 |
| 25 tons of manure | 117 | 180 | 2,540 | 101 |
| 12.5 per cent of water | 100 | 100 | 100 | 100 |
| 15 per cent of water | 90 | 111 | 118 | 108 |
| 17.5 per cent of water | 91 | 113 | 121 | 102 |
| 20 per cent of water | 79 | 123 | 121 | 104 |
| 22.5 per cent of water | 87 | 119 | 123 | 108 |

It will be observed that the manure increases the number of bacteria developing upon the synthetic media, while the water depresses the number developing. In neither case is the regularity as great as could be desired. The ammonifying powers of the soil very regularly increases as the manure applied increases. The increase becomes less each time in a definite quantity as the manure increases. The water causes an increase in the ammonifying powers of the soil up until 20 per cent of water is applied; above this it causes a decrease. It would have been very interesting and practical to have added greater quantities of water to find whether it would have continued to depress the ammonification.

The quantity of nitric nitrogen systematically increases as the water and manure applied increase, and it may be seen, as would be expected, that there is a close correlation between the ammonification and nitrification. The nitrogen-fixing powers regularly increase up to 10 tons of manure per acre; above this they gradually decrease. The water tends

in all cases to increase the nitrogen gained. It will thus be observed that the manure applied increases the bacterial activities measured, while the water increased ammonification, nitrification, and nitrogen fixation, but depressed the number of colonies developing upon synthetic media. This would seem to be a very vital point against the count method. For we thus find a soil treatment increasing the main bacterial activities of a soil, but at the same time depressing the number developing in the laboratory. It would thus appear that the media used to make counts was better adapted for the development of organisms other than those which take the greatest part in the nitrogen transformation in the soil. On the other hand, it is quite possible that the increase in number may not keep pace with the increased physiological efficiency due to the application of water and manure. But this latter explanation would not account for the less number developing on the synthetic media.

## FIELD EXPERIMENT ON FALLOW PLOTS

The fallow plots used in the field experiments were 7 feet wide and 24 feet long with a 4-foot walk between each two. The land was plowed in the fall, left over until spring, when a mixture of fairly well-rotted horse and cow manure was applied to the various manured plots. This was thoroughly disked or plowed into the soil. Water was applied to the plots from flumes as described in Utah Experiment Station Bulletins 115 to 120. They were kept free from weeds throughout the year. The quantities of water and manure applied to the various plots were as follows:

Four plots received no water and no manure.

Two plots received 5 inches of water, but no manure. The water was in two equal applications.

Two plots received 10 inches of water, but no manure. The water was applied in two equal applications.

Two plots received 20 inches of water, but no manure. The water was applied in four equal applications.

Two plots received 30 inches of water, but no manure. The water was applied in six equal applications.

Three plots received 40 inches of water, but no manure. The water was applied in eight equal applications.

All of the above were repeated with plots receiving 5 and 15 tons of manure to the acre. Hence, the series includes soils without manure, with 5 tons per acre, and with 15 tons per acre. The water applied varied from none up to 40 inches both with and without manure. This does not, however, represent the entire water reaching the soil, for there was an average annual precipitation of about 18 inches, most of which fell between the months of October and May. The precipitation from May to November did not exceed 5 inches, which, of course, would be

uniform for all plots. The plots had been treated since the spring of 1911 in the manner described; the bacteriological analyses were made during the summer of 1914 and 1915.

The results reported in Table IV giving the number of colonies of bacteria developing in four days on synthetic agar represent in every case the average of a number of determinations made at the times indicated.

TABLE IV.—*Number of colonies of bacteria developing in four days on synthetic agar—fallow plots*

| Number of determinations. | Treatment. | Number of colonies. | | | |
|---|---|---|---|---|---|
| | | May 12. | July 25. | Nov. 12. | Average. |
| 12 | No water; no manure | 3,475,000 | 12,500,000 | 4,100,000 | 5,692,000 |
| 6 | 5 inches of water; no manure | 3,000,000 | 7,600,000 | 3,700,000 | 4,767,000 |
| 6 | 10 inches of water; no manure: | 2,960,000 | 12,900,000 | 3,700,000 | 6,520,000 |
| 6 | 20 inches of water; no manure | 3,030,000 | 12,600,000 | 3,950,000 | 6,527,000 |
| 6 | 30 inches of water; no manure | 2,370,000 | 15,800,000 | 5,700,000 | 7,957,000 |
| 9 | 40 inches of water; no manure | 5,660,000 | 11,860,000 | 3,800,000 | 7,107,000 |
| 6 | No water; 5 tons of manure | 3,570,000 | 23,500,000 | 4,300,000 | 10,457,000 |
| 6 | No water; 15 tons of manure | 7,700,000 | 20,000,000 | 4,800,000 | 10,833,000 |
| 3 | 5 inches of water; 5 tons of manure | 4,000,000 | 11,800,000 | 6,600,000 | 7,467,000 |
| 3 | 5 inches of water; 15 tons of manure | 5,600,000 | 14,200,000 | 11,200,000 | 10,333,000 |
| 3 | 10 inches of water; 5 tons of manure | 4,600,000 | 19,000,000 | 7,600,000 | 10,400,000 |
| 3 | 10 inches of water; 15 tons of manure | 6,000,000 | 28,000,000 | 4,000,000 | 12,667,000 |
| 3 | 20 inches of water; 5 tons of manure | 4,300,000 | 18,000,000 | 6,600,000 | 9,633,000 |
| 3 | 20 inches of water; 15 tons of manure | 4,400,000 | 27,400,000 | 9,800,000 | 13,867,000 |
| 3 | 30 inches of water; 5 tons of manure | 6,200,000 | 21,200,000 | 4,400,000 | 10,600,000 |
| 3 | 30 inches of water; 15 tons of manure | 3,600,000 | 29,400,000 | 3,200,000 | 12,067,000 |
| 9 | 40 inches of water; 5 tons of manure | 4,450,000 | 14,066,000 | 5,933,000 | 8,150,000 |
| 9 | 40 inches of water; 15 tons of manure | 4,600,000 | 19,933,000 | 6,200,000 | 10,244,000 |

It may be seen that the number of organisms are comparatively low during the spring, in no case exceeding 8,000,000, while in July the number becomes in most cases three or four times as many. In November the number developing is about the same as in May. This method therefore gives a maximum count in midsummer. The spring samples were taken after all frost had left the ground, while the fall samples were taken before

there occurred any very hard frost; consequently these numbers do not in any case represent the numbers found in frozen soil, which would probably be higher than any of the results herein reported.

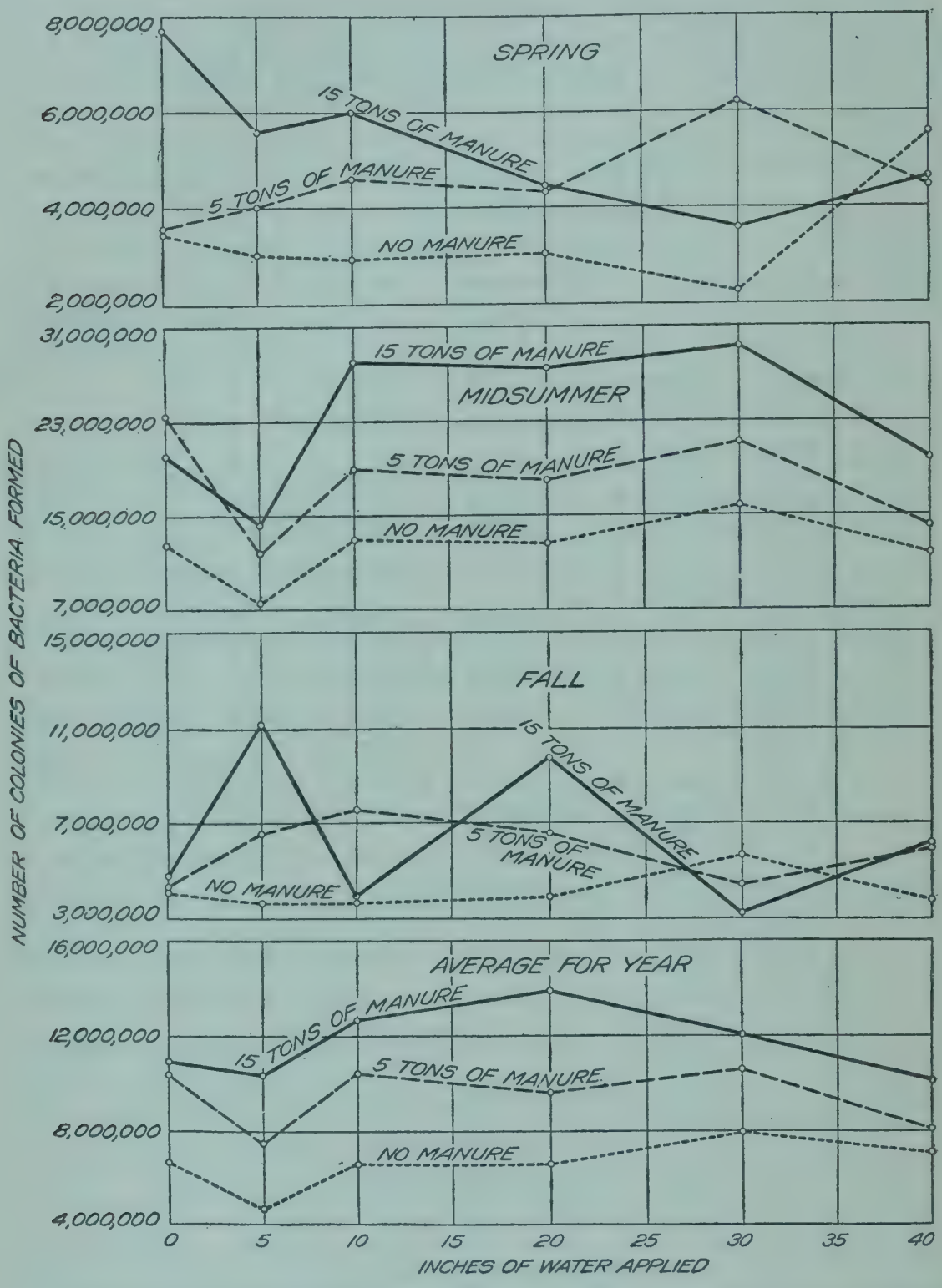


FIG. 3.—Curves of the number of colonies of bacteria developing from fallow soil with varying quantities of manure and water.

The results obtained for May show the unmanured soil to have few bacteria present, while the number in the manured soil increases as the quantity of manure increases. The water apparently had no marked effect upon their activity; or if it had, it had been obliterated during the winter

months. In July much the same order occurs. The soil receiving 15 tons of manure per acre contains more bacteria than that receiving 5 tons, and this in turn has more than the unmanured soil. Here the influence of the water becomes very marked, for there are many more bacteria in the soils receiving 10, 20, or 30 inches of water than in the soils receiving either no water or 40 inches. The excessive quantity of water, 40 inches, apparently checks the development of bacteria on the synthetic media.

The same results, in general, are obtained for November as for May and July, and with the exception of the abnormal results reported, where 10 inches of water were applied, the water has a pronounced effect even as late as November. This difference disappears during the winter, for we find a more uniform condition existing the next spring.

The average results for the unmanured soil show that more bacteria developed from the soil receiving 30 inches of water than from those receiving either more or less irrigation water. The manured soil, on the other hand, gave a maximum count from the soil receiving 20 inches of water. These differences are clearly brought out in figure 3. On the horizontal line is indicated the quantity of water applied, while on the perpendicular is given the number of colonies which developed. At the top of the figure are given the results for the spring, while below this in the order named for midsummer, fall, and the average for the year.

If we consider the average number of bacteria developing on synthetic media from the unmanured plots as 100 per cent, those developing on the manured plots become, with 5 tons of manure, 147 per cent, and with 15 tons, 177 per cent, showing that in so far as numbers are concerned the greatest effect per ton of manure applied is produced by the addition of 5 tons per acre. If we average the unirrigated plots and take these as 100 per cent, the others become, with 5 inches of water, 81 per cent; with 10 inches of water, 106 per cent; 20 inches of water, 107 per cent; 30 inches of water, 110 per cent; and 40 inches of water, 91 per cent. The maximum increase is apparently due to the application of 30 inches of irrigation water. But here, as was the case with the pot experiments, the results are not uniform.

The same plots were tested for ammonification, the results being given in Table V. In every case the result is the average of a number of closely agreeing determinations and are given as milligrams of ammonia produced in four days in 100 gm. of soil containing 2 gm. of dried blood.

The ammonifying powers of the soil, as may be seen from Table V, remain nearly constant throughout the season. There is, however, a big variation in the ammonifying powers of the different soils. In the spring the ammonifying powers of the unmanured soils are low. The quantity of ammonia formed in no case exceeds 57 mgm. per 100 gm. of soil. The water applied apparently had no perceptible influence upon the rate of ammonification. The quantity of ammonia produced by the soil receiving 5 tons per acre of manure is much higher than that pro-

duced by the unmanured, and the addition of water up to 10 inches produces a beneficial effect. The great effect, however, is noted on those soils which receive 15 tons of manure per acre. Here, also, the ammonifying powers are accelerated by the application of irrigation water up to 10 inches. Above this there is a depressing effect just as was noted in the pot experiments and can very likely be accounted for on the same grounds. In midsummer the influence of manure is just as perceptible as it is in the spring, and the influence of the water becomes much more regular, but still follows the same general trend that it did in the spring. In the fall the manure is found to exert almost quantitatively the same effect as it does in spring and midsummer. The depressing effect of the larger quantities of water during this season of the year is not as great as it is earlier in the year. But even here the higher applications (20 to 40 inches) cause a great falling off in the ammonifying powers of both the manured and unmanured soils. These results are brought out graphically in figure 4.

TABLE V.—*Quantity of ammonia (in milligrams) produced in four days in 100 gm. of soil containing 2 gm. of dried blood—fallow plots*

| Number of determinations. | Treatment. | Quantity of ammonia. | | | |
|---|---|---|---|---|---|
| | | May 12. | July 25. | Nov. 12. | Average. |
| 12 | No water; no manure | 56. 38 | 55. 52 | 81. 00 | 64. 30 |
| 6 | 5 inches of water; no manure | 54. 78 | 47. 60 | 77. 10 | 59. 82 |
| 6 | 10 inches of water; no manure. | 50. 99 | 46. 25 | 64. 65 | 53. 96 |
| 6 | 20 inches of water; no manure. | 49. 56 | 44. 65 | 62. 80 | 52. 33 |
| 6 | 30 inches of water; no manure. | 46. 62 | 44. 30 | 61. 70 | 50. 87 |
| 9 | 40 inches of water; no manure. | 49. 87 | 42. 83 | 63. 97 | 52. 22 |
| 6 | No water; 5 tons of manure. | 73. 92 | 71. 00 | 77. 70 | 74. 31 |
| 6 | No water; 15 tons of manure. | 92. 65 | 82. 95 | 82. 25 | 85. 95 |
| 3 | 5 inches of water; 5 tons of manure. | 81. 09 | 58. 30 | 88. 70 | 76. 03 |
| 3 | 5 inches of water; 15 tons of manure. | 116. 55 | 97. 60 | 92. 00 | 102. 05 |
| 3 | 10 inches of water; 5 tons of manure. | 96. 29 | 76. 30 | 93. 80 | 88. 79 |
| 3 | 10 inches of water; 15 tons of manure. | 129. 20 | 118. 50 | 112. 00 | 119. 90 |
| 3 | 20 inches of water; 5 tons of manure. | 86. 79 | 70. 00 | 89. 60 | 82. 13 |
| 3 | 20 inches of water; 15 tons of manure. | 111. 59 | 108. 5 | 106. 6 | 108. 89 |
| 3 | 30 inches of water; 5 tons of manure. | 82. 45 | 70. 40 | 79. 90 | 77. 58 |
| 3 | 30 inches of water; 15 tons of manure. | 112. 63 | ..... | 105. 80 | 109. 2 |
| 9 | 40 inches of water; 5 tons of manure. | 98. 78 | 79. 47 | 90. 50 | 89. 58 |
| 9 | 40 inches of water; 15 tons of manure. | 106. 51 | 91. 63 | 100. 33 | 99. 49 |

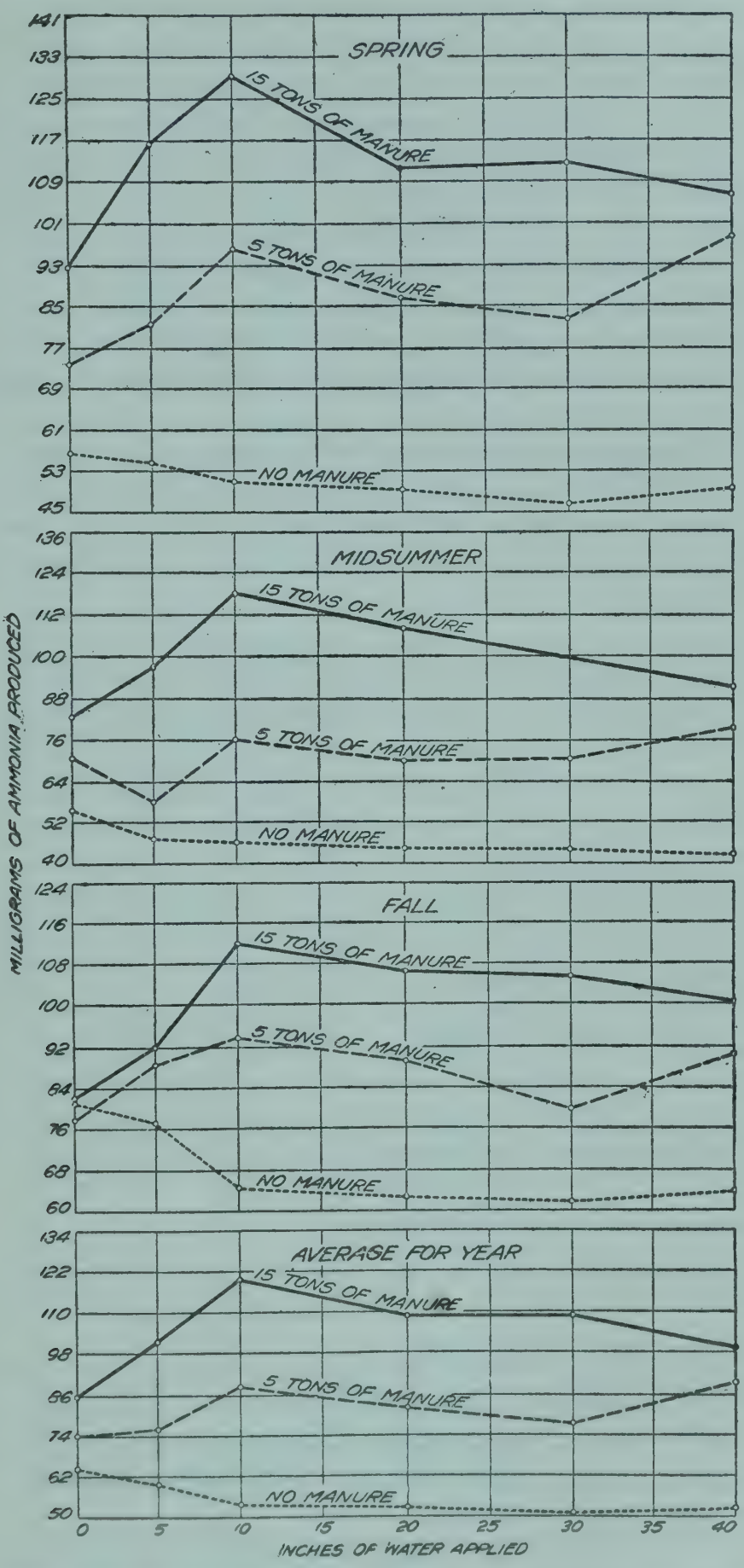


FIG. 4.—Curves of the ammonifying powers of fallow soil with varying quantities of manure and water.

If we take the average quantity of ammonia produced in the unmanured soil as 100 per cent and compare this with that produced in the manured soil, we find those soils receiving 5 tons of manure produce 147 per cent and those receiving 15 tons produce 188 per cent; or the average increase per ton of manure applied is twice as great when 5 tons are applied as when three times that much is used.

Considering the average of the soil receiving no irrigation water as 100 per cent, the others then become with 5 inches of water, 106 per cent; with 10 inches of water, 117 per cent; 20 inches of water, 108 per cent; 30 inches of water, 106 per cent; and 40 inches of water, 108 per cent. The greatest increase in ammonifying powers results from the application of 10 inches of irrigation water.

The nitrifying powers were determined as previously outlined, and the results reported in Table VI represent milligrams of nitric nitrogen formed during 21 days in 100 gm. of soil containing 2 gm. of dried blood. The results as reported are the average in each case of a number of determinations taken during two years.

TABLE VI.—*Quantity of nitric nitrogen (in milligrams) produced in 21 days in 100 gm. of soil to which had been added 2 gm. of dried blood—fallow plots*

| Number of determinations. | Treatment. | Quantity of nitric nitrogen. | | | |
|---|---|---|---|---|---|
| | | May 12. | July 25. | Nov. 12. | Average. |
| 12 | No water; no manure | 1.46 | 16.36 | 2.16 | 6.66 |
| 6 | 5 inches water; no manure | 1.40 | 11.90 | .88 | 4.72 |
| 6 | 10 inches water; no manure | .79 | 13.85 | .97 | 5.20 |
| 6 | 20 inches water; no manure | 1.19 | 13.30 | 1.33 | 5.27 |
| 6 | 30 inches water; no manure | 1.40 | 9.27 | 1.00 | 3.89 |
| 9 | 40 inches water; no manure | 1.05 | 5.37 | .89 | 2.43 |
| 6 | No water; 5 tons of manure | 1.47 | 10.32 | 2.15 | 4.64 |
| 6 | No water; 15 tons of manure | 11.90 | 40.43 | 30.50 | 27.61 |
| 3 | 5 inches water; 5 tons of manure | 4.03 | 4.20 | 7.35 | 5.19 |
| 3 | 5 inches water; 15 tons of manure | 1.75 | 45.20 | 31.85 | 26.27 |
| 3 | 10 inches water; 5 tons of manure | 1.47 | 24.85 | .70 | 9.01 |
| 3 | 10 inches water; 15 tons of manure | 2.63 | 26.25 | 15.40 | 14.76 |
| 3 | 20 inches water; 5 tons of manure | 1.23 | 7.70 | 11.20 | 6.71 |
| 3 | 20 inches water; 15 tons of manure | 2.53 | 40.95 | 18.90 | 20.79 |
| 3 | 30 inches water; 5 tons of manure | .88 | 2.80 | 2.80 | 2.16 |
| 3 | 30 inches water; 15 tons of manure | 2.52 | 21.00 | 46.90 | 23.47 |
| 9 | 40 inches water; 5 tons of manure | 1.19 | 2.33 | 2.22 | 1.91 |
| 9 | 40 inches water; 15 tons of manure | 2.63 | 25.78 | 33.65 | 20.69 |

All of these results will appear low when compared with those obtained by many other workers, who report their results as milligrams of nitrates found. The nitrifying powers of all the soils are low in the spring, but become much higher in midsummer and fall back in autumn to about where they were in the spring.

During the spring the nitrifying powers of the soil vary with the manure applied. But the difference existing between the manured and unmanured soil in no case is great. The irrigation water which had been

applied during the previous season exerted no effect which carried over the winter. In midsummer the nitrifying powers of the soil receiving 5 tons of manure are apparently less than the soil receiving no manure. The plots receiving 15 tons per acre are much more active in nitrifying dried blood than are the others. The lower applications of irrigation water apparently exert a favorable influence on all the plots, but the greater applications exert a depressing influence. It is, however, no more marked in the heavily manured soils than in the others; therefore, if there be any denitrification taking place, it must be attributed to the production of anerobic conditions by the water, and not due to the manure applied. In November the beneficial influence of the 5 tons of manure applied becomes more regular than at any other time of the year. Here also the influence of the water becomes more perceptible. Taking the results as a whole they do not show the influence of either manure or water as well as it was shown by the potted soils; nor do they bring out the difference as clearly as it is brought out by the ammonification series. The relationship actually existing in the various treated soils is brought out graphically in figure 5.

On the base line is indicated the irrigation water applied in inches per acre, while on the perpendicular line is given the milligrams of nitric nitrogen produced in 100 gm. of soil to which 2 gm. of dried blood had been added. Taking the average nitric nitrogen produced in the unmanured soil as 100 per cent, the soil receiving 5 tons of manure becomes 105 per cent, while that of the soil receiving 15 tons becomes 486 per cent; or the average increase per unit of manure applied is much greater when 15 tons of manure are applied than when only 5 tons are applied. In this respect it differs markedly from the ammonification series.

Taking the average of the unirrigated plots as 100 per cent, the irrigated plots then arrange themselves in the order—5 inches, 94 per cent; 10 inches, 75 per cent; 20 inches, 85 per cent; 30 inches, 76 per cent; and 40 inches, 65 per cent. In every case the average for the season on all plots shows the water to have a depressing influence upon nitrification.

## FIELD EXPERIMENTS ON CROPPED PLOTS

The same number of plots, arranged and treated exactly the same as those in the preceding part except that they were cropped, were sampled. These had grown corn continuously since the spring of 1911. They were sampled at the same time of the year, and bacterial counts made as was done on the fallow soil. The average results are given in Table VII.

These results are very similar to those obtained on the fallow soil. The number of organisms obtained is slightly lower and we do not find as great an increase during the summer months as we do on the fallow. The count as obtained in the spring is low for the unmanured soil, higher for that receiving 5 tons per acre of manure and still higher for the soil receiving 15 tons of manure. While the difference is marked,

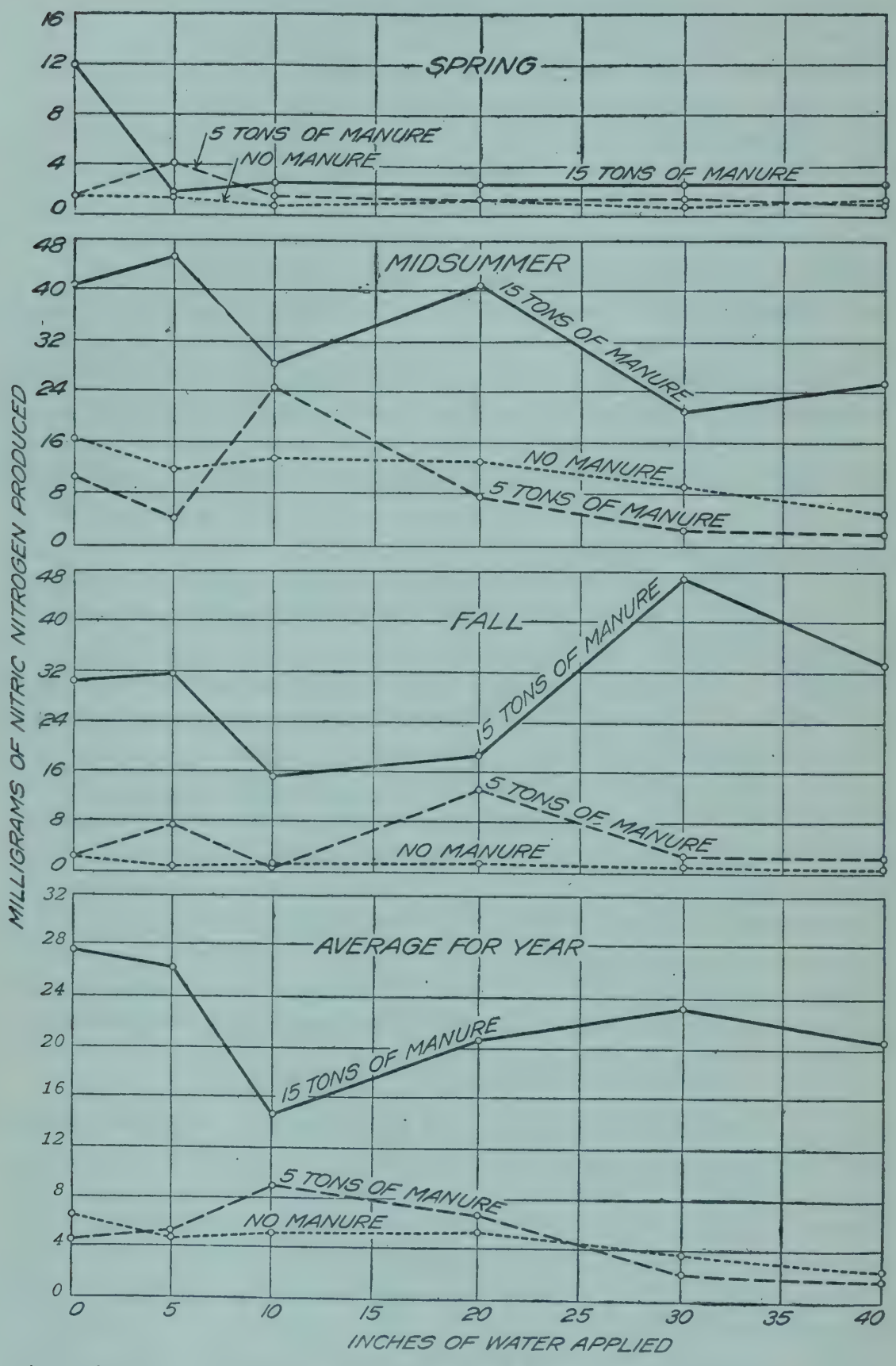


FIG. 5.—Curves of the nitrifying powers of fallow soil with varying quantities of manure and water.

it is not as pronounced as it is in the fallow soil. The same general order is seen during spring and fall, but in the fall the difference is greater in degree and more regular than in the earlier part of the year. The application of irrigation water produces an increase with the lower applications, especially on the heavily manured soil. The irregularity of this set as compared to the fallow can be accounted for in a degree by the error entering in sampling, for in some cases the sample may be taken nearer a plant than in others and in the cultivation and irrigation the tendency would be to leave the soil less homogeneous in the cropped than in the fallow plots. These conditions were borne in mind at the time of sampling and efforts made to get representative samples, but the results show that much more care must be taken on cropped than on fallow soil. The results for this series of plots are given graphically in figure 6.

TABLE VII.—*Number of colonies of bacteria developing in four days on synthetic agar—cropped plots*

| Number of determinations. | Treatment. | Number of colonies. | | | |
|---|---|---|---|---|---|
| | | May 10. | Aug. 9. | Nov. 8. | Average. |
| 6 | No water; no manure | 4,300,000 | 7,300,000 | 4,000,000 | 5,200,000 |
| 6 | 5 inches of water; no manure | 4,500,000 | 4,250,000 | 2,700,000 | 3,817,000 |
| 6 | 10 inches of water; no manure | 5,800,000 | 3,950,000 | 1,800,000 | 3,850,000 |
| 6 | 20 inches of water; no manure | 5,200,000 | 6,150,000 | 1,800,000 | 4,387,000 |
| 6 | 30 inches of water; no manure | 5,100,000 | 4,600,000 | 2,000,000 | 3,900,000 |
| 6 | 40 inches of water; no manure | 4,300,000 | 4,700,000 | 5,700,000 | 4,900,000 |
| 6 | No water; 5 tons of manure | 8,300,000 | 4,700,000 | 3,200,000 | 5,400,000 |
| 6 | No water; 15 tons of manure | 5,300,000 | 5,400,000 | 2,200,000 | 4,300,000 |
| 6 | 5 inches of water; 5 tons of manure | 6,300,000 | 5,300,000 | 2,900,000 | 4,833,000 |
| 6 | 5 inches of water 15 tons of manure | 8,800,000 | 6,950,000 | 6,800,000 | 7,517,000 |
| 6 | 10 inches of water; 5 tons of manure | 6,100,000 | 6,300,000 | 7,800,000 | 6,733,000 |
| 6 | 10 inches of water; 15 tons of manure | 6,800,000 | 5,800,000 | 4,200,000 | 4,933,000 |
| 6 | 20 inches of water; 5 tons of manure | 6,100,000 | 6,350,000 | 4,500,000 | 5,633,000 |
| 6 | 20 inches of water; 15 tons of manure | 5,900,000 | 5,450,000 | 4,600,000 | 5,317,000 |
| 6 | 30 inches of water; 5 tons of manure | 4,200,000 | 6,900,000 | 2,800,000 | 4,633,000 |
| 6 | 30 inches of water; 15 tons of manure | 5,800,000 | 6,900,000 | 3,800,000 | 5,500,000 |
| 6 | 40 inches of water; 5 tons of manure | 7,600,000 | 4,450,000 | 3,500,000 | 5,183,000 |
| 6 | 40 inches of water; 15 tons of manure | 5,100,000 | 7,000,000 | 7,800,000 | 6,633,000 |

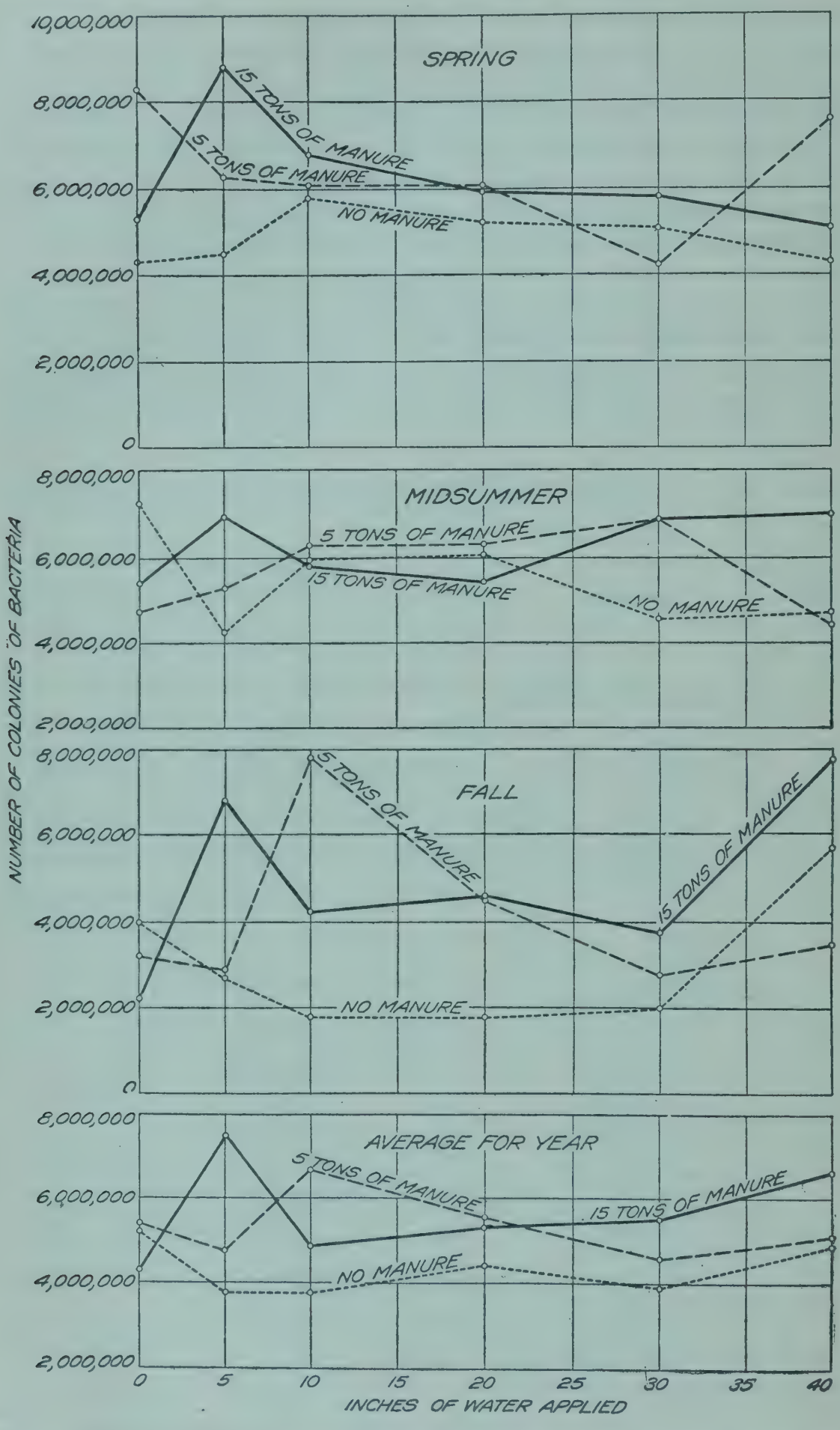


FIG. 6.—Curves of the number of colonies of bacteria developing from cropped plots with varying quantities of manure.

If the average number of bacteria found in the manured soil be taken as 100 per cent, the soil receiving 5 tons of manure then becomes 123 per cent and that receiving 15 tons, 129 per cent. Taking the average bacterial count of the plot receiving no irrigation water as 100 per cent, the others then become with 5 inches of water, 109 per cent; with 10 inches of water, 104 per cent; with 20 inches, 103 per cent; with 30 inches, 94 per cent; and with 40 inches, 112 per cent. With one exception the irrigation water had increased the number of bacteria in the soil.

The same plots were analyzed on the same dates for their ammonifying powers, and the results are given in Table VIII as milligrams of ammonia produced in four days in 100 gm. of soil, to which were added 2 gm. of dried blood. Each result is the average of a number of closely agreeing determinations.

TABLE VIII.—*Quantity of ammonia (in milligrams) formed in four days in 100 gm. of soil containing 2 gm. of dried blood—cropped plots*

| Number of determinations. | Treatment. | Quantity of ammonia. | | | |
|---|---|---|---|---|---|
| | | May 10. | Aug. 9. | Nov. 8. | Average. |
| 6..... | No water; no manure..... | 54.05 | 44.54 | 46.59 | 48.39 |
| 6..... | 5 inches of water; no manure... | 48.96 | 49.64 | 45.73 | 48.11 |
| 6..... | 10 inches of water; no manure.. | 50.10 | 51.17 | 44.54 | 48.60 |
| 6..... | 20 inches of water; no manure.. | 53.04 | 48.27 | 39.95 | 47.09 |
| 6..... | 30 inches of water; no manure.. | 48.96 | 45.05 | 36.89 | 43.63 |
| 6..... | 40 inches of water; no manure.. | 52.87 | 51.55 | 37.07 | 47.16 |
| 6..... | No water; 5 tons of manure..... | 57.80 | 67.15 | 55.25 | 60.07 |
| 6..... | No water; 15 tons of manure.... | 71.69 | 67.15 | 68.85 | 69.23 |
| 6..... | 5 inches of water; 5 tons of manure..... | 60.33 | 60.69 | 53.17 | 58.06 |
| 6..... | 5 inches of water; 15 tons of manure..... | 91.63 | 74.41 | 73.79 | 79.94 |
| 6..... | 10 inches of water; 5 tons of manure..... | 61.08 | 70.69 | 61.54 | 64.44 |
| 6..... | 10 inches of water; 15 tons of manure..... | 92.99 | 89.93 | 87.05 | 89.99 |
| 6..... | 20 inches of water; 5 tons of manure..... | 63.16 | 61.54 | 56.10 | 60.26 |
| 6..... | 20 inches of water; 15 tons of manure..... | 96.69 | 101.15 | 89.45 | 95.76 |
| 6..... | 30 inches of water; 5 tons of manure..... | 57.97 | 68.17 | 51.34 | 59.16 |
| 6..... | 30 inches of water; 15 tons of manure..... | 97.41 | 115.20 | 76.16 | 96.26 |
| 6..... | 40 inches of water; 5 tons of manure..... | 63.07 | 67.49 | 51.01 | 60.52 |
| 6..... | 40 inches of water; 15 tons of manure..... | 86.87 | 91.63 | 77.20 | 85.23 |

The ammonifying powers of these soils are lower, as an average, in the cropped than in the fallow soil. The average quantity of ammonia produced by the fallow soil was 79.43 mgm., while that produced by the cropped soil was 64.48 mgm. The variation due to seasonal differences is not as great in the cropped as in the fallow soil, thus indicating that the influence of the season on the rate of ammonification is greatly offset

by crop and cultural methods. The variation between the differently treated soils during the same part of the year is qualitatively similar to that noted in the fallow soil.

The influence of the manure is very pronounced throughout the entire season. The ammonifying powers of the unmanured soils are all low, while those of soils receiving 5 tons of manure per acre are higher. Those of soils receiving 15 tons of manure per acre are very high. This difference is probably slightly greater during the spring months than during the fall.

The irrigation water applied is found to exert an influence upon this group of bacterial activities. Measured in terms of ammonification, the unmanured soils and those receiving 5 tons of manure per acre are benefited greatly by small quantities (10 and 20 inches) of irrigation water, while the soils receiving 15 tons of manure per acre have the highest ammonifying powers when they receive 20 or 30 inches of water. During the spring it is greatest in those soils from plots receiving 30 inches of irrigation water. Forty inches of water produce a marked depression in the ammonia formed, being pronounced in the soils receiving 15 tons of manure not only in the cropped soil but also in the fallow and potted soils. It is clear, therefore, that large quantities of water applied to a soil rich in organic matter depress the beneficial bacterial activities of that soil. The fallow unmanured soils and soils receiving 5 tons of manure per acre showed a slight decrease in the ammonifying powers of the soil, owing to the larger applications of irrigation water; but this does not appear in the cropped soil and is probably caused by the removal of large quantities of water by the growing crop, so that enough water does not accumulate in the presence of these small quantities of organic material to injure the ammonifying powers of the soil. These facts are brought out clearly in figure 7.

If we take the average of the quantity of ammonia produced in the unmanured soil as 100 per cent, the others then become with 5 tons 129 per cent and with 15 tons 183 per cent. Here the average increase per ton of manure applied is about the same whether 5 or 15 tons of manure be applied per acre. If the average of the plots receiving no irrigation water be taken as 100 per cent, the others then become with 5 inches of water 105 per cent; with 10 inches, 114 per cent; 20 inches, 118 per cent; 30 inches, 112 per cent; and 40 inches, 109 per cent. It thus reaches its maximum when 20 inches of water are applied, while the fallow reached its maximum when only 10 inches were applied. This is a difference which is undoubtedly due to the great quantities of water removed by the growing plant. The average increase per acre-inch of water, however, is greatest in the cropped soil where only 10 inches of irrigation water were applied.

The nitrifying powers of the same soils were tested by the method previously given, the results of such tests being given in Table IX as milligrams of nitric nitrogen produced during 21 days in 100 gm. of

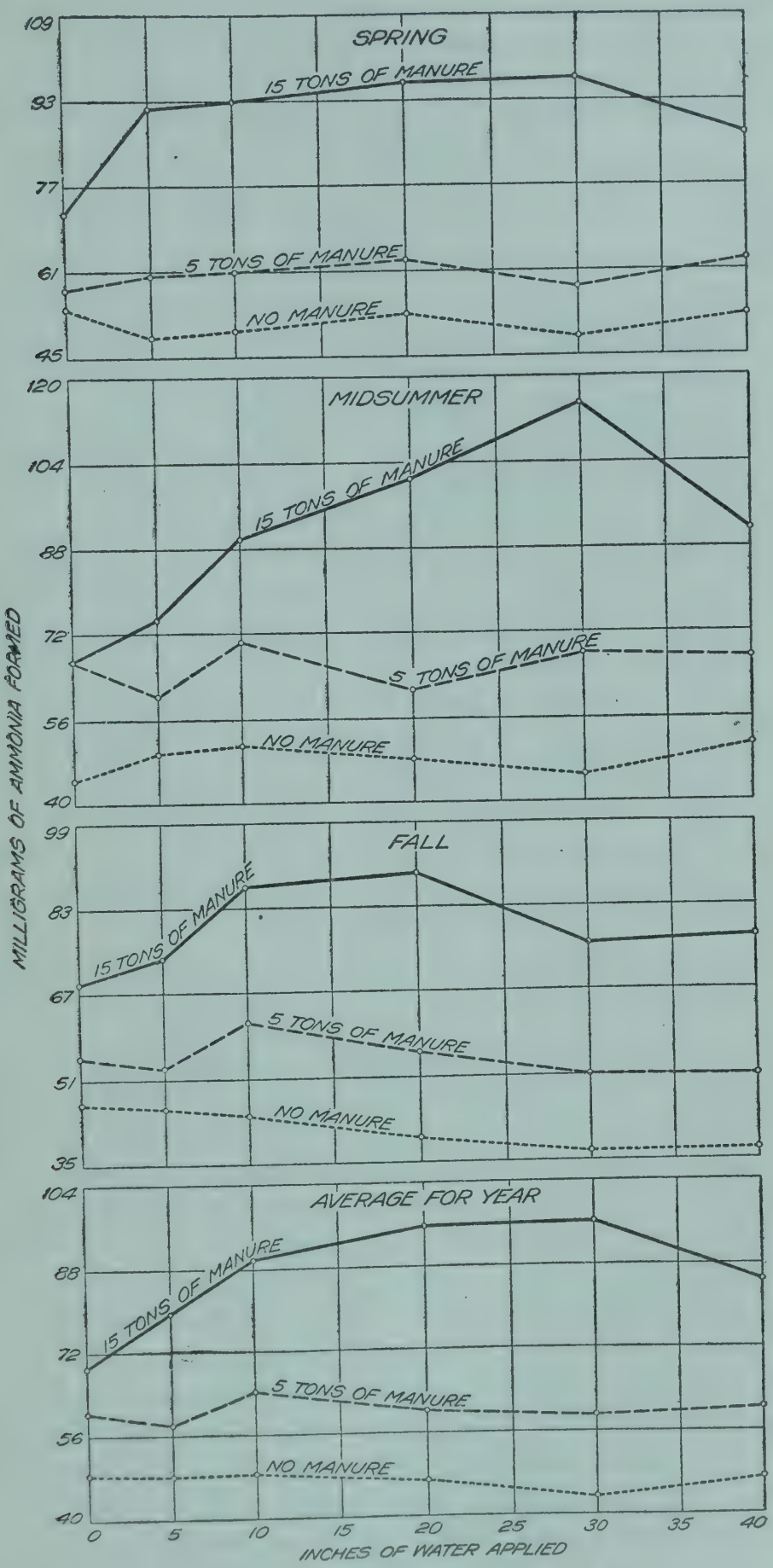


FIG. 7.—Curves of the ammonifying powers of soil of cropped plots with varying quantities of manure and water.

soil containing 2 gm. of dried blood. All the reported results are the average of two or more closely agreeing determinations.

TABLE IX.—*Quantity of nitric nitrogen (in milligrams) formed in 100 gm. of soil containing 2 gm. of dried blood*

| Number of determinations. | Treatment. | Quantity of nitric nitrogen. | | | |
|---|---|---|---|---|---|
| | | May 10. | Aug. 9. | Nov. 8. | Average. |
| 6..... | No water; no manure..... | 1.50 | 1.33 | 0.84 | 1.22 |
| 6..... | 5 inches of water; no manure.... | 3.47 | .81 | .88 | 1.72 |
| 6..... | 10 inches of water; no manure.. | 2.27 | .56 | .53 | 1.12 |
| 6..... | 20 inches of water; no manure.. | 1.05 | .50 | 1.85 | 1.13 |
| 6..... | 30 inches of water; no manure.. | 1.40 | .38 | .35 | .71 |
| 6..... | 40 inches of water; no manure.. | 2.10 | .70 | 1.15 | 1.31 |
| 6..... | No water; 5 tons of manure..... | 1.65 | 1.80 | 2.45 | 1.97 |
| 6..... | No water; 15 tons of manure.... | 45.32 | 8.85 | 27.52 | 27.23 |
| 6..... | 5 inches of water; 5 tons of manure..... | 20.30 | .53 | 3.43 | 8.08 |
| 6..... | 5 inches of water; 15 tons of manure..... | 46.80 | 2.66 | 33.65 | 27.70 |
| 6..... | 10 inches of water; 5 tons of manure..... | 4.90 | .58 | 8.48 | 4.65 |
| 6..... | 10 inches of water; 15 tons of manure..... | 47.45 | 2.27 | 22.92 | 24.21 |
| 6..... | 20 inches of water; 5 tons of manure..... | 9.27 | .63 | 2.66 | 4.18 |
| 6..... | 20 inches of water; 15 tons of manure..... | 53.05 | 2.17 | 23.80 | 26.34 |
| 6..... | 30 inches of water; 5 tons of manure..... | 12.30 | .40 | 2.80 | 5.17 |
| 6..... | 30 inches of water; 15 tons of manure..... | 60.25 | 15.46 | 4.93 | 26.88 |
| 6..... | 40 inches of water; 5 tons of manure..... | 18.37 | .45 | 6.75 | 8.52 |
| 6..... | 40 inches of water; 15 tons of manure..... | 37.05 | 1.01 | 14.08 | 17.38 |

The nitrifying powers of these soils are uniformly higher in the spring months of the year than later. This occurs in all the plots, but the greatest difference is found in the heavily manured plots, due probably to the application of large quantities of readily nitrifiable material in the manure, which is transformed later into soluble nitrates taken up by the growing plant, removed in the drainage water, or transformed into complex protein substances within the bodies of various microorganisms. The results taken as a whole bear a very great similarity to those obtained on the fallow soil. They are, however, as were the counts and ammonifying powers, slightly higher in the fallow than in the cropped soil.

The nitrifying powers of the unmanured soil are low throughout the year. The nitrates produced by the manured soil increase with the increase of manure applied. The greatest difference, however, exists between the soil receiving 5 and 15 tons of manure per year. In the latter the nitrifying activity is extremely active in the spring months. This difference, while not as pronounced later in the year, exists throughout the season.

The irrigation water exerts a great influence upon the nitrifying powers of the soil and this follows almost exactly the order followed by the ammonifying series. It is greatest when a medium amount of water is applied, but becomes injurious as greater quantities of water are applied to the soil, especially with large quantities of organic matter. One could not conclude from these results that the quantities of water here applied in the presence of organic manure favor denitrification, but it is certain that the conditions thus produced are not the best for the nitrate and ammonia-forming organisms, and it is quite likely due to the anerobic condition produced by the excess of water. It is interesting to note that larger quantities of water are required on a cropped soil to exert this depressing influence than on a fallow soil. The results for this series are given graphically in figure 8.

Taking the average quantity of nitric nitrogen produced in the unmanured soil as 100 per cent, the soil receiving 5 tons of manure then becomes 453 per cent, while the percentage produced in the soils receiving 15 tons per acre becomes 2,079. Thus, an enormous increase is due directly to the application of manure to the soil.

Taking the average quantity of nitric nitrogen produced in the soil receiving no irrigation water as 100 per cent, the irrigated soils produced with 5 inches of water, 126 per cent; with 10 inches of water, 99 per cent; 20 inches of water, 104 per cent; 30 inches of water, 108 per cent; and 40 inches of water, 89 per cent—an unmistakable reduction in the nitrifying powers of soils receiving 40 inches of irrigation water.

## RELATIONSHIP IN BACTERIAL ACTIVITIES IN POTTED, CROPPED, AND UNCROPPED SOIL

If we use in every case the quantity of ammonia and nitric nitrogen produced and the total number of bacteria developing from the unmanured in the one case and the unirrigated in the other as 100 per cent, we have a direct comparison between the bacterial activities of the variously treated soils. The results so obtained are given in Table X.

TABLE X.—*Comparison of the bacterial activities in the potted, fallow, and cropped soils*

| Treatment. | Bacteria. | | | Ammonia. | | | Nitric nitrogen. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pots. | Fallow. | Cropped. | Pots. | Fallow. | Cropped. | Pots. | Fallow. | Cropped. |
| | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* | *Per ct.* |
| No manure | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 5 tons of manure | 99 | 144 | 123 | 122 | 147 | 129 | 1,211 | 105 | 453 |
| 15 tons of manure | 100 | 177 | 129 | 152 | 188 | 183 | 2,240 | 486 | 2,079 |
| No irrigation water | [a] 100 | 100 | 100 | [a] 100 | 100 | 100 | [a] 100 | 100 | 100 |
| 5 inches of water | [b] 90 | 81 | 109 | [b] 111 | 106 | 105 | [b] 118 | 94 | 126 |
| 10 inches of water | [c] 91 | 106 | 104 | [c] 113 | 117 | 114 | [c] 121 | 75 | 99 |
| 20 inches of water | [d] 79 | 107 | 103 | [d] 123 | 108 | 118 | [d] 121 | 85 | 104 |
| 30 inches of water | [e] 87 | 110 | 94 | [e] 119 | 106 | 112 | [e] 123 | 76 | 108 |
| 40 inches of water | ..... | 91 | 112 | ..... | 107 | 108 | ..... | 65 | 89 |

[a] 12.5 per cent applied.
[b] 15 per cent applied.
[c] 17.5 per cent applied.
[d] 20 per cent applied.
[e] 22.5 per cent applied.

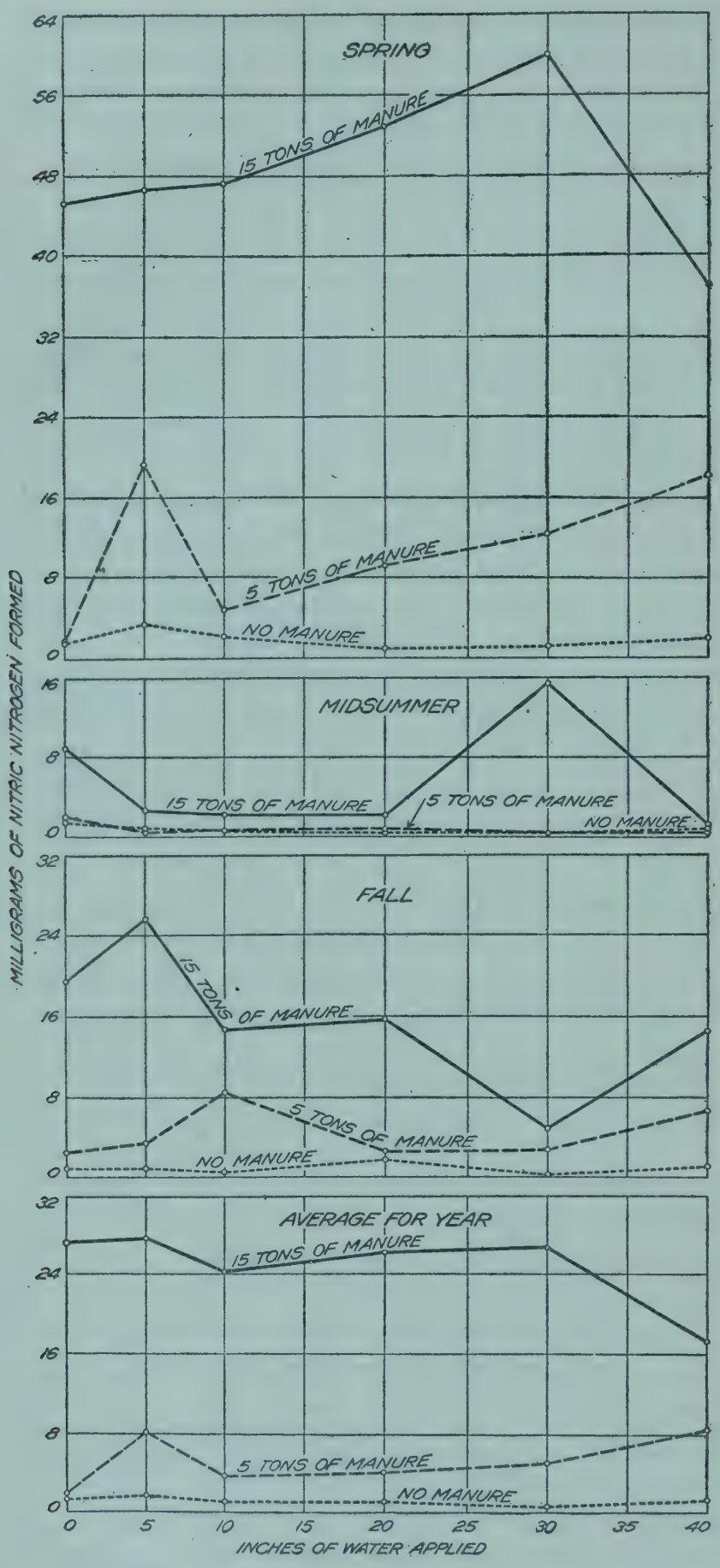


FIG. 8.—Curves of the nitrifying powers of soil of cropped plots with varying quantities of manure and water.

The results for manure show a remarkable uniformity throughout. With one exception it has increased the bacterial count and also the bacterial activities of the soil, and this is about the order throughout. The ammonifying and bacterial counts are increased more by the manure in the fallow than in the cropped soil.

The irrigation water applied apparently increases the bacterial count in the fallow and cropped field soil but it apparently depresses it in the potted soil. The ammonifying powers of all soils are uniformly increased with increasing amounts of irrigation water applied up to a certain application. Above this there is a depression. Greater quantities of water must be applied to cropped than uncropped soil in order to cause this depression. This is mainly owing to the influence of the plant upon the moisture content of the soil.

The nitrifying powers of the potted soils are very uniform in showing a beneficial effect due to the water. The cropped soil is not so uniform, while the fallow soil shows a depressing influence. These apparently contradictory results are quite likely caused by a difference in treatment, for the water in the three different sets of soil may have been far from the same.

## RELATIONSHIPS BETWEEN BACTERIAL ACTIVITIES AND CROP-PRODUCING POWERS

The results herein reported, together with those published by Dr. Harris (19) upon Greenville soil, make it possible to compare directly the crop produced on the soil as an average of five years with the bacterial activities of the soil. This is done in figures 9 and 10, in which the bacterial activities and crop-producing powers of the unmanured soil are taken as 100 per cent and each of the manured plots compared with this. In the case of water applied the bacterial activities and crop produced upon the soils receiving no irrigation water are taken as 100 per cent and the others compared with this.

An examination of figure 9 shows a remarkably close correlation between the crop produced and the bacterial activities of the soil. The extent to which the bacterial count and ammonifying powers of the soil are increased by the manure applied is almost quantitatively the same as the increase in the crop produced on the manured soil. The increase in the nitrifying powers of the soil is much greater than the crop increase due to manure, but they are all of the same order.

An examination of figure 10 reveals the fact that the application of 5 inches of irrigation water increases in nearly the same proportion the crop produced and the bacterial activities of the soil. The average percentage for the crop is 112, while the total average bacterial activities is 113 per cent. The crop produced on the soil receiving 10 inches of water is slightly less than that produced on the soil receiving 5 inches

of irrigation water. With the exception of the ammonia produced, the bacterial activities are not as high in the soil receiving 10 as in the soil receiving only 5 inches of irrigation water. The average percentage of the crop produced on this is 110, while the average of the bacterial activities is 102 per cent. The application of 20 inches of irrigation water greatly increased the crop produced and also the bacterial activities, the crop produced being 127 per cent compared with the unirrigated, while using the same comparison for bacterial activities gives 108 per cent. The application of 30 inches of irrigation water causes a slight decrease in the corn produced and also in the bacterial activities of the soil, 40 inches of irrigation water producing about the same crop as did 30 inches. But it caused a slight falling off in the bacterial activities of the soil, especially in the nitrifying powers of the soil. Taking the result as a whole, we find that the bacterial activities of the soil and the crop-producing powers of a soil are both influenced by the application of irrigation water and this in the same direction and in about the same degree. These results tend to indicate that the bacteriological analysis of a soil gives a fair insight into its relative crop-producing powers, being especially true with regards to the ammonifying and nitrifying powers of the soil.

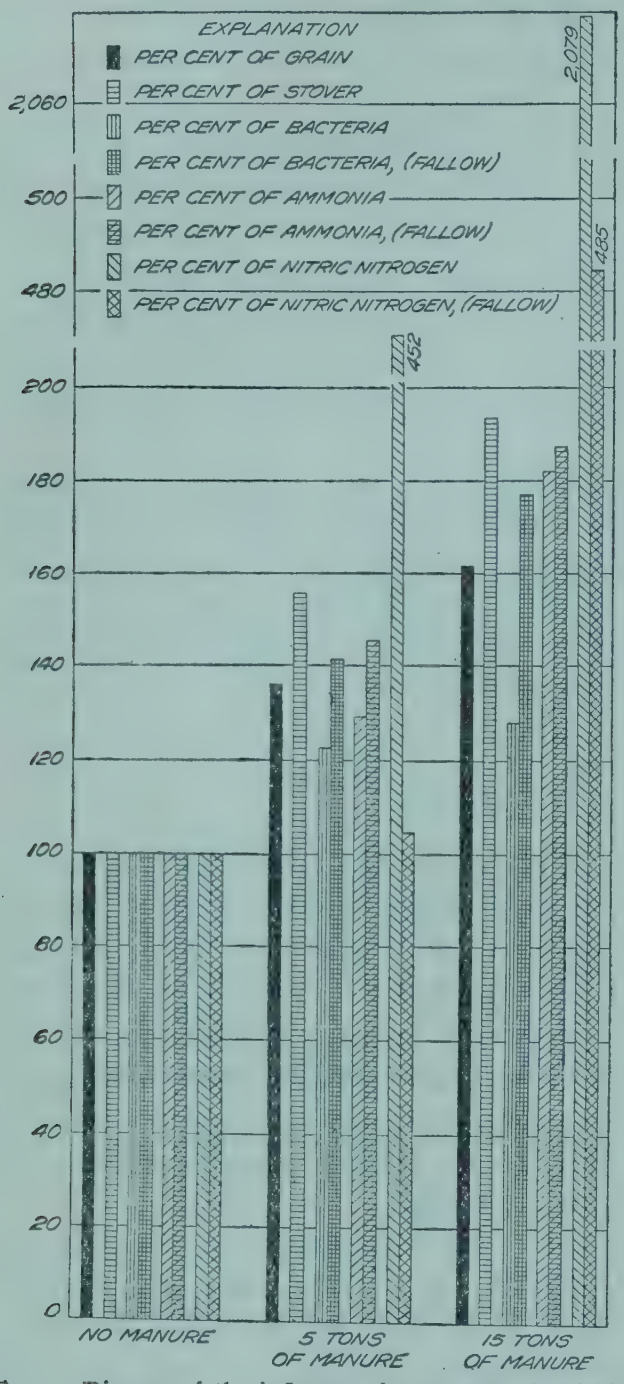


FIG. 9.—Diagram of the influence of manure on the yield and bacterial activities of a soil, the unmanured plots being expressed as 100 per cent.

## SUMMARY

A calcareous soil kept in pots with varying amounts of manure and different percentage of moisture gave on bacteriological analyses at the end of four months the following results.

The temperature of the manured and unmanured averaged practically the same for the period, but the temperature of the soil with 12.5 per cent of water averaged 1 degree centigrade higher than did soils with 22.5 per cent of water. The greatest number of organisms developed on synthetic media from the soils receiving the greatest quantity, 25 tons, of manure. There were more colonies developed from the soil receiving 12.5 per cent of water than from any of the other soils receiving higher quantities of water.

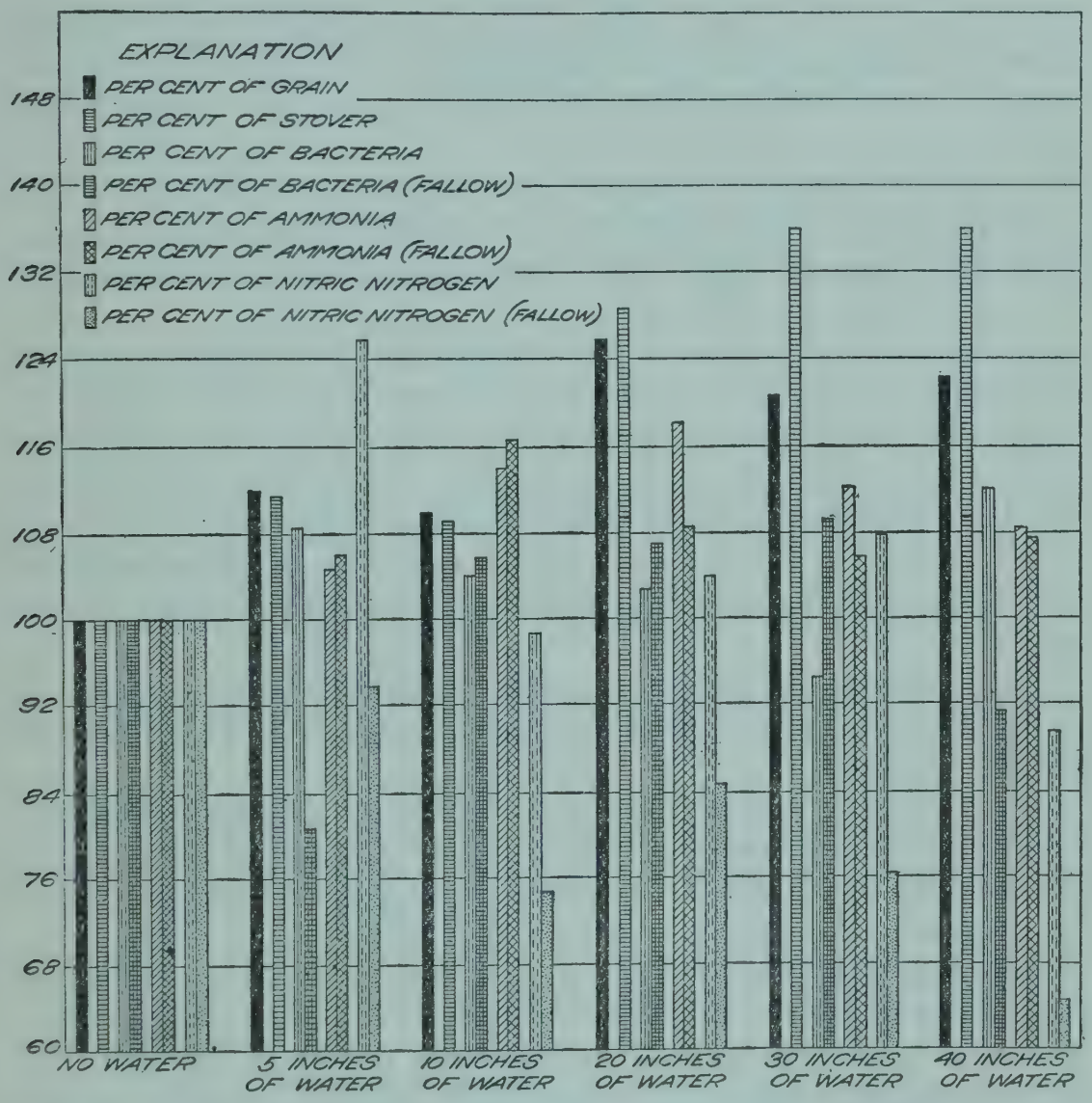


FIG. 10.—Diagram of the influence of irrigation water on the yield and bacterial activities of a soil, the nonirrigated plots being expressed as 100 per cent.

The ammonifying powers of the soil increased with the manure applied up to 25 tons of manure per acre, but the greatest increase per ton of manure was obtained in soil receiving 5 tons.

The ammonifying powers of the soils increased as the water applied increased until 20 per cent of water was applied. The ammonifying powers of soil receiving 22.5 per cent of water were not as high as were those of soil receiving 20 per cent of water. The greatest increase per unit of water applied was when the water was increased from 12.5 to 15 per cent of water.

The nitrifying powers of the soil increased as the manure and water applied increased up to 25 tons of manure and 22.5 per cent of water.

The nitrogen-fixing powers of the soil were greatest in those pots receiving at the rate of 10 tons of manure per acre. Increasing the water above 12.5 per cent but not above 22.5 per cent slightly increased the nitrogen-fixing powers of the soil. Nothing in the results indicated that the application of manure up to 25 tons per acre and of water up to 22.5 per cent caused denitrification in the soil.

Bacteriological analyses of fallow field soil receiving none, 5 tons, and 15 tons of manure per acre and receiving none, 5 inches, 10 inches, 20 inches, 30 inches, and 40 inches of irrigation water gave the following results.

The maximum number of bacteria were obtained from the soil receiving 15 tons of manure. The application of irrigation water up to 20 inches increased the bacterial count, being most noticeable in the soil receiving the greatest quantity of manure.

If the ammonifying powers of the unmanured soils are considered as 100 per cent and the unirrigated as 100 per cent, the manured and irrigated soils then become with 5 tons of manure, 147 per cent; with 15 tons of manure, 188 per cent; 5 inches of water, 106 per cent; 10 inches of water, 117 per cent; 20 inches of water, 108 per cent; 30 inches of water, 106 per cent; and 40 inches of water, 108 per cent. Large quantities of irrigation water produced the greatest depressing effect in the presence of 15 tons of manure per acre.

The application of manure to a soil increases its nitrifying powers. The application of irrigation water to a fallow soil apparently depresses its nitrifying powers.

Fewer organisms develop on synthetic agar from a cropped than from a fallow soil. The application of manure to a cropped soil increases the bacterial count of the soil. The greatest number of organisms developed from the soil receiving 10 inches of irrigation water.

The ammonifying powers of the cropped soils were slightly lower than similarly treated fallow soils. The application of 5 and 15 tons of manure per acre to a soil increases the ammonifying powers of the soil. The application of irrigation water up to 30 inches increases the ammonifying powers of the soil. The greatest increase resulted in those soils receiving 15 tons per acre of manure. The application of 40 inches of irrigation water to corn land, especially to that receiving 15 tons of manure per acre, depresses the ammonifying powers of the soil.

The nitrifying powers of fallow soil were higher than similarly treated cropped soils. The application of manure to a cropped soil greatly increases its nitrifying power. The application of irrigation water up to 30 inches, especially to a soil receiving 15 tons of manure per acre, greatly increases its nitrifying powers.

There was found to be a direct relationship between the bacterial count, the ammonifying powers, the nitrifying powers, and the crop produced on a soil receiving no manure, 5 tons, and 15 tons of manure per acre.

A close correlation was also found to exist between the bacterial activities of soil receiving varying amounts of water and crop produced upon the soil.

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# PROGRESSIVE OXIDATION OF COLD-STORAGE BUTTER

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## OUTLINE OF PREVIOUS WORK

Much has been written concerning the changes occurring in butter. The word "change" is here used in its broad and general sense to include any perceptible alteration whatsoever, although it refers principally to an organoleptic one, whether induced by one or several factors.

Butter has been kept for certain periods of time during which it has been exposed to the action of various decomposing and disintegrating agencies, and a study of the products of change thereby resulting has led investigators to draw conclusions relative to the causation of the "off flavors" so often found in stored butter. As a general rule, the majority of opinions advanced in accounting for the deterioration of butter seem to have been based either upon insufficient analytical data or upon a study of butter or butter fat kept under conditions which prevail only to a very limited degree when butter is stored.

Many investigators confined their attention to a study of the fat of butter alone and sought to attribute the appearance of undesirable flavors in whole butter to some change which this one constituent undergoes. However, more recent investigations carried on with fats other than butter would appear to render such an assumption doubtful and would seem to make imperative more conclusive information concerning the causation of disagreeable flavors in whole butter held in cold storage.

The early literature in regard to the chemical changes which take place in butter is voluminous, but it is also conflicting and confusing, a great deal of it being of a purely speculative nature.

A great variety of bodies, products of chemical change, have presumably been identified in butter kept under varying conditions. The confirmation of the presence after a certain interval of time of such substances in fats known to have been originally pure is of value; yet such data obtained in the investigation of a material containing other constituents as well are obviously not so satisfactory unless it is definitely known that these attendant components do not likewise undergo

similar changes. Acids, aldehydes, alcohols, and esters, among other things, may have been identified in spoiled fats, and even up to the present time it has been customary to attribute their origin solely to the fat itself. The reason for such deduction is evident. It is well known that the fat of butter is in itself a most complex material. It is a composite, made up of mixtures of the glycerids of fatty acids. Among the saturated glycerids butyrin is an essential ingredient, although palmitin and myristin predominate. Olein has generally been considered to be the only unsaturated glycerid in butter fat, yet quite recently Laxa and Konecny (3)[1] claim to have found that the fatty acids of the "liquid fat" of separator slime consist of 49.65 per cent of erucic acid and 21.24 per cent of oleic acids; but this assumption may not be entirely justified.

The improbability of any chemical change occurring in the saturated glycerids of storage butter is quite generally recognized; consequently the glycerid olein, purely because it contains an unsaturated linkage in the molecule and absorbs the halogens with avidity, has been considered as the source from which are derived those decomposition products the presence of which in fats influences their more or less decreased value. As a matter of fact, any satisfactory and conclusive evidence that the olein of butter fat is readily susceptible to oxidation under conditions similar to those prevailing when butter is stored is entirely lacking. On the other hand, it has been demonstrated that pure olive oil, the liquid glycerids of which consist almost entirely of olein, shows very little absorption of oxygen as measured by the iodin number, even after having been kept for three years under ordinary conditions (5). Masters and Smith (6), in preliminary experiments with butter fat, found but little change in the iodin value during cooking experiments carried out with this material. To obtain any pronounced change in the iodin value and in the acidity, they found it necessary to heat their samples of butter fat to as high a temperature as 200° C. while passing oxygen through the material, the mere heating of the fat to such temperature under ordinary conditions proving to be insufficient. From these two illustrations, as well as from more recent work done by other investigators, the discussion of which owing to limited space is omitted, it must be concluded that the possibility of the olein of butter fat undergoing an appreciable oxidation caused by the small quantity of atmospheric air inclosed in a package of butter is very remote, especially when it is remembered that butter is stored in the dark at a temperature considerably lower than the freezing point of water.

The inability of chemists to judge the quality of an edible fat because of the absence of satisfactory chemical data has been frequently pointed out, and this is attributable primarily to the lack of appropriate and comprehensive analytical procedure. For instance, rancidity has generally been regarded as the natural concomitant of acidity, yet a pronounced

[1] Reference is made by number to "Literature cited," p. 951.

rancidity may have appeared in a stored fat without the manifestation of any increased acidity as measured by a simple titration. Again, an undue significance may be attached to a slight decrease in the original iodin number of the fat. Such a decrease is usually considered to be caused by the taking up of oxygen by the double bond of the unsaturated glycerid; yet it must be remembered that self-polymerization—the interlocking of two or more molecules of the unsaturated glycerids—may occur, a condition which would likewise bring about a lowering of the iodin value. Again, the olein of butter fat may not exist entirely as the normal glycerid, and it is possible that a certain amount of this glycerid may occur as an isomerid. So far as is known to the writer no work has been carried out to determine whether the olein of stored butter is present entirely as the normal glycerid. In this connection it may be observed that the work of Ponzio and Gastaldi (8) and of Fokina (1) indicates that the farther the double bond is removed from the carboxyl group the nearer the iodin number approaches the theoretical value. Normal oleic acid gave the theoretical value of 90. On the contrary, 2–3 oleic acid gave a Hübl number of only 6.6, Wijs 20.4, Hanus 1.9. While there are no data at hand at present to prove that 2–3 oleic acid actually does occur in butter fat, yet this contingency is quite possible; and it is well to take it into consideration as yet another factor which may produce a slight lowering of the iodin number of stored butter. It is evident, however, that the customary methods in vogue to determine the quality of fat leave much to be desired.

One of the factors so often construed as influencing the appearance of undesirable flavors in a fat is the nature of the impurity, or impurities, contained therein. In just what manner these foreign substances bring about these undesirable characteristics has not been fully cleared up, because it is conceivable that it depends upon several parallelly progressing chemical reactions and because it is possible that slight chemical changes really difficult of identification by analytical methods suffice to produce the above-mentioned disagreeable features.

It is apparent that even at the present time there seems to be considerable doubt as to whether the undesirable flavors of storage butter arise from a decomposition occurring in the fat itself or in some one or more of the other components entering into the composition of the whole product. For this reason it is thought advisable to confine the preliminary work on this subject to an attempt to settle this most basic consideration before proceeding with the further investigation of the causation of the "off flavors" so frequently met with in storage butter.

## STATEMENT OF THE PROBLEM AND METHOD OF SOLUTION

Even in those times when the chemical constitution of the fats was still unknown it had been surmised that the changes which oils and fats underwent on keeping were simply the result of oxidation. This is the

view most generally held at the present time, and the more recent literature on the subject indicates that this phase of research is to be continued with no less abated interest. It is still unknown whether the development of undesirable flavors in storage butter is dependent upon an oxidation occurring in the fat itself or whether the milk sugar and nitrogenous constituents of the curd are those components of the butter most susceptible to oxidation. Approximately 10 per cent of the volume of butter is air (9), and it is quite possible that, owing to the oxygen of the air inclosed within the material, a slight and progressive oxidation may take place in the interior of a package of butter. This possibility, when considered together with the known fact that marked and undesirable alterations in the flavor of butter during storage may be brought about by acidifying the pasteurized cream from which the butter is made (10), has suggested the idea that an examination of the air inclosed within packages of butter differently prepared and in butter fat alone might furnish some interesting data as to whether the undesirable chemical changes occurring in stored butter are caused by a progressive oxidation in the fat itself or in some one or more of the nonfatty ingredients.

It was deemed advisable to pursue this line of investigation in a manner not previously attempted, so far as known. Samples of pasteurized sweet-cream butter, butter made from pasteurized cream to which lactic acid had been added, and butter made from pasteurized cream to which a starter had been added and which was churned at once, were prepared, packed in glass tubes, and stored. Tubes from each lot were removed from storage after certain intervals of time had elapsed and an analysis of the air therefrom was made by means of the gas apparatus specially designed for the purpose. (See fig. 1 and Pl. CXI.) It was hoped that the analytical data so obtained would show some distinguishing features between the three samples dissimilarly prepared, especially with respect to the sample made from acid cream. It was also decided to make use of the determination of the chemical constants of the pure butter fat to serve merely as an indication as to whether any chemical alteration of the fat through oxidation had occurred during the storage interval, confirmed by the analysis of the air extracted from packages of butter fat to determine whether the oxygen content therein is diminished during the storage period. The data so obtained were used as a standard, and the aim kept in view was to study the effect, if any, of the presence of varying amounts of nonfatty constituents (protein, lactose, etc.) upon the decomposition of the fat of butter and, in addition, to note whether the presence of varying quantities of these substances in the butter induced an alteration during storage in the composition of the air incorporated in the samples at the time of their manufacture. Samples of pure butter fat and of butter containing varying quantities of buttermilk

were also prepared, packed into tubes, and stored under the same conditions as the foregoing samples. The effect of a large amount of air upon a small quantity of butter fat and upon buttermilk containing varying quantities of acid was studied by filling other tubes with pumice fragments which were then impregnated with fat or buttermilk and an analysis of the air therefrom made after certain intervals in storage had elapsed.

## DESCRIPTION AND MANIPULATION OF THE GAS APPARATUS USED

In figure 1 is depicted the apparatus constructed for use in the extraction and analysis of the air confined in the packages of the various

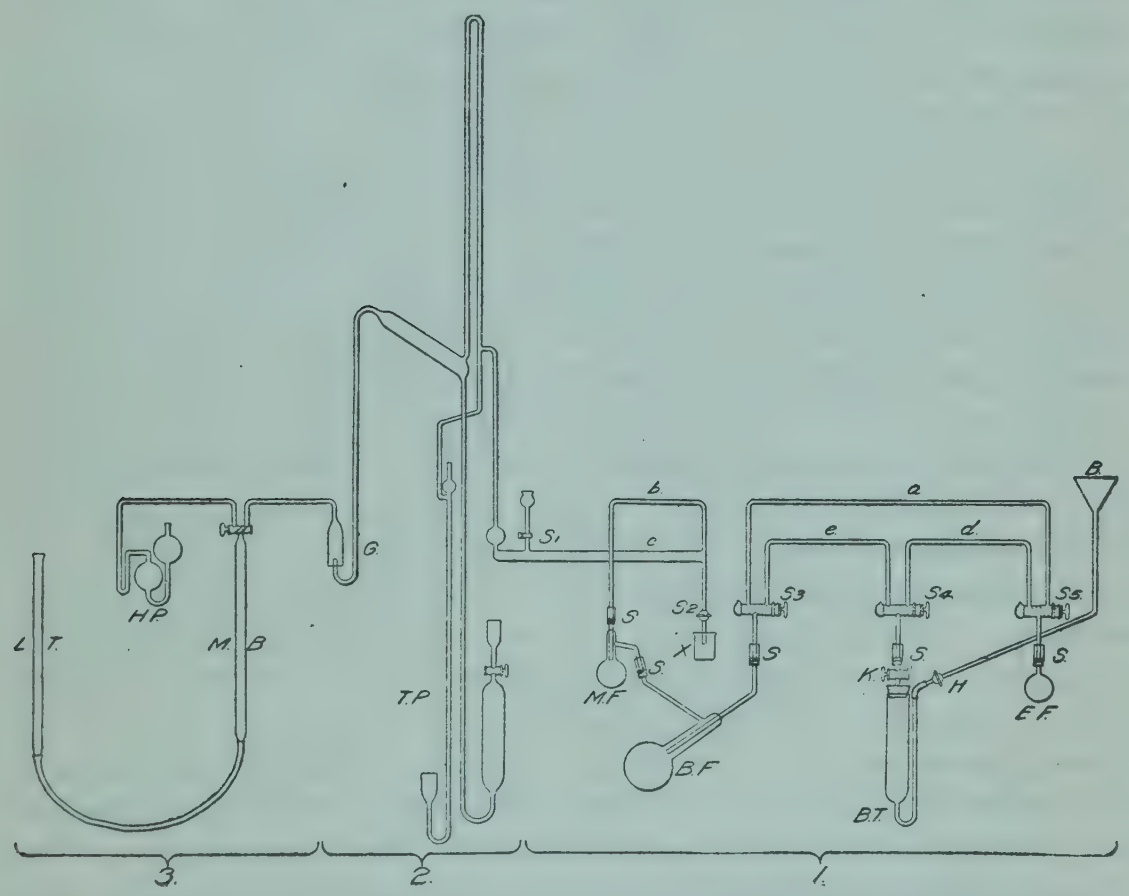


FIG. 1.—Diagram of gas apparatus used in the extraction and analysis of the air confined in butter.

samples of butter fat and butter put up and stored for the investigation which has been described. The apparatus is of glass throughout and consists of three divisions: (1) The system for extracting the gas from the butter tubes, (2) the Töpler pump for transferring the gas so obtained to (3) the usual Hempel apparatus. The rigid and undetachable arrangement of glass tubing and mercury-seal stopcocks comprising the upper part of 1 is conveniently fastened to a wooden frame by means of small, brass pipe bands in the manner seen in Plate CXI, which shows the entire apparatus set up for use. The lower, detachable parts of the extracting system (see fig. 1) consist of the butter tube B. T., the construction and nature of which are described later and which contains the sample

under investigation; the butter flask B. F., of about 1 liter capacity, for retaining the sample after its passage through part of the system; the moisture flask M. F., of 200 c. c. capacity, for retaining the greater part of the moisture liberated from the sample; and a small evacuation flask or globe, E. F., of about the same capacity. These detachable parts are connected with the upper part of the system by means of various mercury seals, S, S.

The operation is begun late in the afternoon of the day before the actual determination by putting the entire division 1 under vacuum and allowing it to stand in this condition overnight. This is done in the following manner:

With the exception of the butter tube B. T., the apparatus is connected as illustrated: The mercury-seal stopcocks $S_1$, $S_2$, and $S_4$ are closed and stopcocks $S_3$ and $S_5$ are so turned as to open the system from E. F. through *a*, B. F., M. F., *b*, and *c* to the Töpler pump T. P. The Töpler pump is now given one stroke, which serves somewhat to exhaust the air confined in the system; a small beaker, *x*, filled with concentrated sulphuric acid is brought under stopcock $S_2$ so that the tube projecting downward from the stopcock is plunged well beneath the surface of the acid, and the beaker is supported in this position. Stopcock $S_2$ is now cautiously opened until the acid rises to form a long level of drying agent covering the bottom of tube *c*, when the influx of acid is stopped. The pump is now worked to its limit of exhaustion (about 0.3 mm. on the McLeod gauge). A turning back and forth of stopcock $S_5$ accompanied with successive strokes of the pump will evacuate tube *d* to stopcock $S_4$, and this is followed by turning stopcock $S_3$ and working the pump to exhaust the tube *e*. The entire division 1 is now under exhaust and is allowed to remain so for a considerable length of time, preferably overnight. The next morning, if the gauge indicates that no leakage of air into the system has occurred, the actual determination is made as follows:

The moisture flask M. F. is covered by a beaker which is then packed with cracked ice and salt (sodium chlorid). The butter tube B. T. is connected at $S_4$ by means of a mercury seal. Funnel B is closed at stopcock H, and filled with a three-fourths saturated sodium-chlorid solution at a temperature of 50° C. A little of this brine solution is allowed to trickle from a pipette into the small side tube of the butter tube B. T. until the latter is completely filled, whereupon it is connected with the funnel tube below H by means of a piece of tight-fitting rubber vacuum tubing. A large glass jar (not shown in the illustration) is now used to cover the butter tube B. T., the base of which rests upon a large rubber stopper with its center removed. The mercury-seal stopcock $S_5$ is now turned to connect the evacuation flask E. F. with *d*, and a turn of $S_4$ toward *d* followed by a closing of the same serves to evacuate the tube from $S_4$ to the glass stopcock K of the butter tube B. T. $S_3$ is opened to connect *e*

with the system B. F., etc. The stopcocks $S_4$, K, and H are now all closed. Water at a temperature of 45° C is poured into the glass jar surrounding B. T. until it immerses the rubber stopper carrying the stopcock K and the rubber connection between the small side tube to B. T. and H. The warmth thus applied to the butter tube at once causes a slight pressure against K and H. H is opened first to allow one or two trapped bubbles of air to escape up toward B and is then closed. K is immediately opened and is soon after followed by the opening of H again. As the material in B. T. melts, a graduated and regulated opening of $S_4$ permits most of the air confined within the sample to pass over into the system, and the remaining air follows with the melted fat, etc., which passes up, around, and down through *e* and trickles into B. F. A too rapid passing of butter containing much curd should be prevented, as it will cause considerable foaming in the butter flask. The warm salt solution flowing in from B displaces the sample from B. T. When the material has been thus removed from B. T. and the level of the salt solution has reached $S_4$, this stopcock is closed, followed also by the closing of $S_3$. The gas is now transferred from the apparatus by the Töpler pump to the gas-collecting tube G, allowing a few minutes to elapse between strokes of the pump, thus permitting the gas containing moisture not removed by M. F. to become dried by passing over or remaining in contact with the sulphuric acid in *c*. The gas is collected over mercury in G and is drawn therefrom into the mercury-filled measuring burette M. B. connected with the leveling tube L. T., from which it is passed over into the Hempel pipettes H. P., where the quantities of carbon dioxid and oxygen in the gas are determined in the usual manner with solutions of potassium hydroxid and alkaline pyrogallol.

## SPECIAL BUTTER TUBES[1]

These tubes are about 9 inches long and 1¾ inches in diameter, with necks widened somewhat to accommodate a No. 9 rubber stopper carrying a glass stopcock. An ordinary-sized glass tube, bent on itself, leads upward from the base. Each of these tubes when packed will contain about 250 gm. of butter.

These tubes were cleaned, sterilized, and packed with the sample, allowing a very small air space between the surface of the butter and the rubber stopper. Pure, neutral, paraffin oil was poured on the surface of the butter and the stopper was pressed in until the oil in the tube had risen above the stopcock. The stopper was wired down tightly and the stopcock closed. A few cubic centimeters of paraffin oil were then allowed to flow down the side tube. Butter packed in this manner is free from contact with the outside air.

[1] The use of these tubes for packing and storing butter was suggested by Mr. L. A. Rogers, of the Dairy Division.

## EFFECT OF CREAM ACIDITY UPON THE COMPOSITION OF THE AIR IN BUTTER HELD IN STORAGE

The samples, the gas-analysis data of which are given in Tables I, II, and III, were prepared under conditions as nearly identical as possible, the butter having been made at Troy, Pa. In each case the butter was made from 60 pounds of cream taken from one lot, pasteurized at 140° F., and cooled to 48° F. In all three cases the temperature of the buttermilk was 58° F., the quantity of salt added to the butter each time was 12 ounces, and each working was carried to 15 revolutions.

The cream of sample 1 was churned sweet. To the cream of sample 2 was added 15 per cent of the starter, and the churning done at once. Before churning the cream of sample 3, sufficient lactic acid was added to it to make its acidity 0.71 per cent (calculated as lactic acid) by titration.

TABLE I.—*Analysis of air extracted from sweet-cream butter*

[Calculated to 0° C. and 760 mm. Acidity of cream as lactic acid, 0.11 per cent; salt, 1.21 per cent; curd, 0.58 per cent]

| Number of bacteria per gram.[a] | Time stored. | | | Oxygen. | Carbon dioxid. |
|---|---|---|---|---|---|
| | At 0° F. | At 32° F. | At room temperature. | | |
| | *Days.* | *Days.* | *Hours.* | *Per cent.* | *Per cent.* |
| 9,050,000 | 0 | 0 | 0 | [b] 25. 15 | [b] 2. 89 |
| | 0 | 2½ | 1 | 22. 23 | 4. 51 |
| | 0 | 15 | 1 | 15. 96 | 7. 58 |
| | 0 | 41 | 1 | 9. 86 | 11. 91 |
| | 0 | 57 | 1 | 5. 49 | 15. 24 |
| | 81 | 0 | 1 | 25. 51 | 1. 49 |
| | 81 | 1 | 1 | 22. 70 | 2. 02 |
| 132,000 | 81 | 13 | 1 | 20. 45 | 2. 86 |
| | 110 | 0 | 5 | 20. 62 | 2. 85 |
| | 110 | 1 | 1 | 23. 00 | 2. 73 |
| | 150 | 0 | 2 | 24. 18 | 1. 62 |
| | 180 | 0 | 1 | 25. 11 | 0. 57 |

[a] Thanks are due Mr. L. A. Rogers, of the Dairy Division, for the bacteriological work in connection with this investigation.

[b] Analysis of gas extracted from butter as soon as tube was packed.

Samples 1 and 2 were shipped on the afternoon of the same day, arriving in Washington, D. C., shortly before noon of the following day, when the butter was immediately packed into sterilized special glass tubes and small jars and then placed in storage at 0° F. Sample 3 was finished late in the afternoon of the same day on which the preceding samples were made, and did not reach Washington until the second morning after, when the butter was at once packed into tubes and jars and placed in storage under the same conditions as above.

From each of the three samples several tubes were taken and transferred to storage at 32° F. The remainder of the tubes were allowed to continue at a storage temperature of 0° F. From time to time tubes were removed from both temperatures, the gas removed therefrom by

means of the specially devised apparatus, and the quantities of carbon dioxid and oxygen determined.

A perusal of Table I discloses the fact that very little alteration occurred in the composition of the air inclosed in this sample of sweet-cream butter made from cream having an acidity of 0.11 per cent when it was kept for about 6 months at a temperature of 0° F. During this interval practically no diminution of the original oxygen content took place, and the only apparent change to be noted is a probable decrease in the small quantity of carbon dioxid which was known to be present in the butter at the time it was made. An appreciable and progressive change did occur, however, when the butter was kept for nearly two months at a temperature of 32° F. In this case it will be noted that the original oxygen content decreased, while there was a corresponding increase in the initial quantity of carbon dioxid.

Every effort was made to keep the tubes containing this sample, as well as those containing the other differently prepared samples, under comparable conditions. In this connection it may be mentioned that, since it is necessary to surround the tubes with warm water (45° C.) to melt the butter sufficiently to cause it to flow through the apparatus used and that this procedure if carried out immediately upon the removal of the tubes from storage might result in cracking them, the plan was adopted of allowing them to warm up slightly at room temperature for one hour, except in two cases, in which the tubes were intentionally permitted to remain a longer period at room temperature for the purpose of obtaining additional information. It was found that a tube of this butter, when allowed to remain for 5 hours at room temperature after a storage period of 110 days at a temperature of 0° F., contained less oxygen than a corresponding tube of the same sample kept for the same length of time at a temperature of 0° F., and for 1 day at a temperature of 32° F. In measuring the effect of raising the storage temperature to 32° F. on a sample which had been stored at 0° F. for 81 days, it is of interest to note that after holding for 1 day at the higher temperature there is a measurable decrease in the quantity of oxygen known to be present in the sample after the 81 days at the lower temperature, and this effect on the same sample is much more pronounced after holding at the higher temperature for an additional 12 days, or a total of 13 days.

It may be concluded, therefore, that sweet-cream butter prepared as this sample was and containing a considerable number of bacteria will show but little alteration in the composition of the air inclosed in it when it is kept for six months at a temperature of 0° F. A perceptible change, however, occurs when the butter is kept at a temperature of 32° F., and a very noticeable one when it is kept at room temperature. The sample of butter used scored 92 when made and 91 at the end of three months. After a period of six months in storage at a temperature of 0° F. the score was given at 90, there being no trace of any undesirable flavor.

TABLE II.—*Analysis of air extracted from butter made from sweet cream churned immediately after the addition of 15 per cent of a commercial starter*

[Calculated to °o C. and 760 mm. Acidity of cream as lactic acid, 0.25 per cent; salt, 1.19 per cent; curd, 0.59 per cent]

| Number of bacteria per gram. | Time stored. | | | Oxygen. | Carbon dioxid. |
|---|---|---|---|---|---|
| | At 0° F. | At 32° F. | At room temperature. | | |
| | *Days.* | *Days.* | *Hours.* | *Per cent.* | *Per cent.* |
| 680, 000 | 0 | 7 | 1 | 10. 89 | 26. 44 |
| | 40 | 0 | 1 | 11. 64 | 25. 35 |
| | 55 | 0 | 1 | 10. 84 | 22. 34 |
| 160, 000 | 80 | 0 | 1 | 10. 70 | 21. 87 |
| | 100 | 1 | 1 | 10. 95 | 20. 92 |
| | 150 | 15 | 1 | 8. 78 | 19. 27 |
| | 205 | 0 | 1 | 9. 00 | 19. 50 |

After the addition of a starter the acidity of the cream from which the butter of sample 2 was made was a little more than twice that of the cream used in the preparation of the sample of sweet-cream butter. A slight but appreciable decrease in the oxygen content of the sample during storage at a temperature of 0° F. was observed, while a perceptible decrease in the carbon dioxid was also manifested. After the sample had been kept for a period at a temperature of 0° F., the effect upon the composition of the air in the butter after standing for several days at a temperature of 32° F. was tabulated, as shown in Table II. This table also shows that a sample of butter made in this manner displays, so far as the composition of the air inclosed in it is concerned, a comparatively slight variation from that observed in the previous case of sweet-cream butter, when both samples are stored at a temperature of 0° F. This sample of butter scored 92 when made, 90 after three months' storage at a temperature of 0° F., and 89 after six and one-half months' storage at the same temperature, there being practically no variation in the flavor during the interval.

The addition of lactic acid to the cream of butter sample 3 before churning brought the total acidity to nearly three times that of the cream used to prepare the foregoing butter of sample 2, and about six and one-half times that of the cream used in making the sweet-cream butter. A pronounced decrease, greater than that observed in either of the two previously given samples of butter, occurred in the oxygen and carbon-dioxid content, even when the butter was stored at a temperature of 0° F., and this decrease was still more marked when it was allowed to remain at a temperature of 32° F. The score of this butter, originally 93, fell to 88 after three months in storage at a temperature of 0° F., and at the end of this interval it had an unclean flavor which was still more pronounced after a period of six months' storage at the same temperature, when the score was 84.

TABLE III.—*Analysis of air extracted from butter made from sweet cream churned immediately after the addition of lactic acid*

[Calculated to 0° C. and 760 mm. Acidity of cream as lactic acid, 0.71 per cent; salt, 0.85 per cent; curd, 0.55 per cent]

| Number of bacteria per gram. | Time stored. | | | Oxygen. | Carbon dioxid. |
|---|---|---|---|---|---|
| | At 0° F. | At 32° F. | At room temperature. | | |
| | *Days.* | *Days.* | *Hours.* | *Per cent.* | *Per cent.* |
| 2,050 | 0 | 6 | 1 | 21.58 | 11.20 |
| 0 | 0 | 32 | 1 | 20.53 | 11.08 |
| 0 | 0 | 48 | 1 | 16.70 | 6.74 |
| 0 | 0 | 62 | 1 | 14.93 | 3.86 |
| 0 | 0 | 80 | 1 | 5.95 | 4.45 |
| 0 | 0 | 82 | 1 | 4.17 | 4.54 |
| 0 | 75 | 0 | 1 | 17.30 | 4.48 |
| 0 | 104 | 0 | 1 | 16.94 | 1.79 |
| 0 | 140 | 0 | 1 | 11.74 | 1.75 |
| 0 | 202 | 0 | 1 | 10.84 | 1.54 |

Having now determined that the decomposition caused by cream acidity progresses at a temperature of 0° F. in a package of butter and can be measured by an analysis of the gas extracted therefrom, the next step in the investigation of the problem concerning the development of "off flavors" in storage butter involved a series of experiments the purpose of which was to determine whether this measurable decomposition occurs in the fat of the butter itself, in the buttermilk, or in both.

## OXIDATION OF PURE BUTTER FAT [1]

The butter fat used in the following determinations was prepared to exclude, so far as possible, by melting, filtering, and washing all ingredients of the butter other than fat and was made from the same lot of cream as the samples of butter $B_1$ and $B_2$, mentioned later. The butter was warmed in a glass vessel to from 32° to 34° C. and allowed to stand, to separate the fat from the greater part of the nonfatty substances. The supernatant fat was then siphoned off, filtered into water at 12° to 14° C., and then thoroughly agitated to granulate it. The fat was then washed several times, salted, and worked on a table worker to the extent of 40 revolutions. The butter fat so prepared was found to contain but 0.05 per cent of protein (total N×6.38). It was packed in absolutely clean and sterile glass jars and also in the special glass tubes for air analy-

[1] The term "pure butter fat" is merely relative. Osborne and Mendel (7) have affirmed that butter fat prepared by centrifugalizing melted butter and pipetting off the clear fat was "entirely free from nitrogen and phosphorus and was devoid of any ash-yielding or water-soluble components." Funk and Macallum (2) have recently challenged this statement as regards nitrogen, since they find that butter fat prepared according to Osborne and Mendel's directions yields easily measured quantities of nitrogen in each of the repeated washings with dilute acid and they conclude that it is very difficult and perhaps impossible completely to free butter fat from nitrogenous substances. McCollum and Davis (4) state that their experiments with butter fat tend to strengthen the conclusion drawn by Funk and Macallum regarding the difficulty of completely freeing the butter fat from nitrogen.

sis. The fat in the jars was covered with a thin layer of paraffin to exclude any action of the atmosphere other than that contained within the material itself. All samples were kept under the same conditions in cold storage at a temperature of 0° F. Samples taken from the lot packed in jars were at once analyzed and, in addition, were scored by Messrs. Corneliuson and Rabild, of the Dairy Division. After intervals of approximately one month, samples were withdrawn from storage, analyzed, and scored. This was continued for several months, during which time a sufficient period had elapsed for the samples to manifest any change which might occur in butter stored for a reasonable length of time.

As may be seen by reference to Table IV, it is certain that no alteration in this sample of butter fat was manifested by the flavor. These samples of nearly pure butter fat showed no physical alteration of any kind after six months or even after one and one-half years in cold storage. There was no development of any characteristic flavor whatsoever, the scoring indicating what might have been expected in case of a material deprived of nearly all its essential ingredients other than fat.

TABLE IV.—*Scores of butter fat stored at 0° F.*

| Age. | Score. | Remarks. | Scorer. |
|---|---|---|---|
| *Months.* | | | |
| 1 | 88 | Oily, clean flavor | Corneliuson. |
| 2 | 87 | do | Do. |
| 3 | 87 | do | Rabild. |
| 4 | 87 | do | Do. |
| 5 | 87 | do | Do. |
| 6 | 87 | do | Do. |
| 18 | 87 | do | Do. |

As noted earlier in this paper, the following determinations were made to establish a standard as a criterion for judging any change which might occur in the fat of the same lot of butter (whole butter) prepared with varying quantities of nonfatty ingredients (TableV).

TABLE V.—*Chemical constants of the butter fat after being nearly freed from the nonfatty ingredients by melting, filtering, and washing and stored at 0°F.*[1]

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| Initial | 30.03 | 37.30 | 226.8 | 5.552 | 87.54 | 3.703 | 0.456 |
| 2 months | 30.17 | 37.42 | 226.8 | 5.572 | 88.10 | 3.785 | .468 |
| 3 months | 29.84 | 36.58 | 226.9 | 5.483 | 87.52 | 3.634 | .427 |
| 4 months | 29.67 | 36.68 | 226.4 | 5.140 | 87.22 | 3.340 | .408 |

[1] The determinations of chemical constants of the fat incorporated were made by Dr. E. G. Thomssen, formerly of the Dairy Division.

These figures would seem to indicate that very little, if any, chemical change occurred in the fat after having been kept in storage at a temperature of 0° F. for a period of four months, and it was so apparent that no pronounced change could be expected until a longer time had elapsed than is usually practiced in storing butter that this experiment was discontinued. It was apparent from the analysis of the fat that no noteworthy oxidation had occurred therein while the experimental samples were held in storage. An analysis of the air confined within the butter fat is given in Table VI.

TABLE VI.—*Analysis of the air in butter fat, stored at 0°F., after being nearly freed from the nonfatty ingredients by melting, filtering, and washing*

[Protein, 0.05 per cent; total N ×, 6.38; calculated to 0° C. and 760 mm.]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| *Months.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 2 | 33.20 | 0.99 | 2.98 | 6.43 | 19.37 | 6.44 |
| 3 | 27.35 | .94 | 3.44 | 5.22 | 19.09 | 5.28 |
| 4 | 29.51 | .93 | 3.15 | 5.94 | 20.13 | 5.72 |
| 5 | 38.90 | 1.38 | 3.55 | 7.63 | 19.61 | 7.51 |
| 12 | 30.81 | 1.76 | 5.71 | 4.27 | 13.86 | 5.81 |
| 24 | 31.59 | .93 | 2.94 | .93 | 2.94 | 6.13 |

[1] After deducting the figure for carbon dioxid from total quantity of gas extracted from the tube, and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

Practically all the carbon dioxid present in the gas extracted from these samples was evidently either in the butter fat at the time of its manufacture or was produced therein within a period of two months after being put into storage. Although the figures would seem to indicate a slight progressive increase in its amount during the storage interval, yet its total amount is small; and in view of the oxygen data obtained it seems to bear little or no relation to the oxygen content. It is very clear, however, that no appreciable oxidation of the nearly pure fat itself occurred during a storage interval of five months; and it was not until after the sample had remained in storage for one year that a slight, measurable oxidation was indicated. In this connection it is thought advisable to note the following general consideration:

Although the iodin numbers obtained for the first and second months and those obtained for the third and fourth months are so close as to resemble duplicate determinations, yet we will take it for granted that the total decrease in the iodin number during the entire period of the investigation is attributable exclusively to the absorption of oxygen by the olein of the fat and not to some one or more of the other factors which, as already indicated earlier in this paper, may influence the data obtained for the iodin number. If we regard 0.72 (the difference between 37.30 and 36.58) as representing the taking up of oxygen by the olein of the fat, the following calculations, based upon this hypothesis, will serve to point out the great improbability of any change in the fat from oxidation during storage at a temperature of 0° F.

Each tube containing the butter fat under investigation in the gas analysis held about 250 gm. of material, corresponding to about 200 gm. of pure fat.[1] The decrease in the quantity of iodin absorbed by a tube would be 1.44 gm., indicating that the fat had absorbed 0.091 gm., or 63.7 c. c. of oxygen. The total quantity of gas incorporated into the sample for the third month, for instance, was only 27.1 c. c., containing approximately but 5.28 c. c. of oxygen in all, and this is obtained from the tube in undiminished quantity in the gas analysis. After one year's storage the material had absorbed only 1.54 c. c. of oxygen, and even after two years' storage the presence of unabsorbed oxygen could still be determined. From the foregoing it will be seen that it is very improbable that any oxidation of pure butter fat occurs during storage at a temperature of 0° F. when the fat is stored for a reasonable length of time. It was decided, however, to make an additional experiment in order to be more certain on this point.

### BUTTER FAT EXPOSED TO A LARGE SURFACE OF AIR

A sample of butter fat was prepared in the same manner as was the preceding material—by melting, filtering, and washing. In addition, it was given a thorough agitation on the shaking machine with four successive changes of warm water containing 0.5 per cent of hydrochloric acid. The warm butter fat so prepared was allowed to flow through the side tube of the special butter tube filled with pumice fragments until it overflowed through the glass stopcock at the top. The tube was then inverted and the butter fat in the tube permitted to run out. In this manner a small quantity of fat, clinging to the pumice fragments, was exposed to the action of a large quantity of air, a condition just the reverse of that in the previous case. The tubes were then stored at 32° F., a temperature considerably higher than that used in the previous cases. The results are given in Table VII.

TABLE VII.—*Oxidation of pure butter fat exposed to the action of a large surface of air at 32° F.*

[Calculated to 0° C. and 760 mm.]

| Age. | Total gas. | Total carbon dioxid.. | | Total oxygen. | | Calculated oxygen.[2] |
|---|---|---|---|---|---|---|
| *Days.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 30 | 98. 60 | 0 | 0 | 19. 50 | 19. 78 | 19. 72 |
| 61 | 90. 30 | 0 | 0 | 16. 32 | 18. 07 | 18. 06 |
| 100 | 89. 40 | 0 | 0 | 15. 98 | 17. 87 | 17. 88 |

[1] Eight tubes of butter fat were put up for this investigation. The average weight of material in each tube was 249.5 gm. The butter-fat content of each tube was approximated as follows: This butter fat was prepared to represent normal butter minus the nonfatty constituents (protein, lactose, etc.). The effort was made to incorporate the average quantity of water into it, and salt also was added. With 1 per cent of salt and 16 per cent of water (the maximum) in the butter fat, 250 gm. of the material in the tube would consist of 2.5 gm. of salt, 40 gm. of water, and 207.5 of fat ("about 200 gm."). Taking any smaller percentage of water than 16 would increase the percentage of fat, which would, of course, call for a greater absorption of iodin than 1.44 gm., expressing the taking up of a greater quantity of oxygen than 63.7 c. c. This would have the effect of making still more pronounced the point here brought out.

[2] After deducting the figure for carbon dioxid from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

From the above-mentioned data it will be seen that but a slight oxidation of the fat occurred during a storage interval of more than three months at a temperature of 32° F., even when the sample was kept under conditions decidedly more favorable, in comparison with the preceding one, to permit any pronounced oxidation. It would appear, therefore, that any oxidation of pure butter fat kept in storage at a temperature of 0° F. for a reasonable length of time, if it occurs at all, must be extremely slight. The results of experiments already conducted, however, have shown that a progressive oxidation in whole butter may occur while held in storage at a temperature of 0° F.

The question, therefore, arises whether there occurs an oxidation progressing in some one or more of the nonfatty constitutents of whole butter. In the attempt to clear up this point the following experiments were conducted.

## OXIDATION OF NONFATS

The butter samples used in the two following experiments were made in the experimental creamery at Troy, Pa., from the same lot of cream as was the preceding sample of butter fat stored at a temperature of 0° F. The cream was pasteurized in a continuous pasteurizer at a temperature of 165° F., and was ripened with a pure culture. The acidity of the cream at the time of churning was 0.40 per cent (calculated as lactic acid). The butter in the churn was washed until the wash water was just clear. One half of the butter in the churn was removed and was designated as "normally washed butter." The other half, which remained in the churn, was now given an additional copious washing in four changes of water and designated as "excessively washed butter."

The sample designated in the experiments as "unwashed butter" was prepared from a different lot of cream, which was pasteurized and ripened under the same conditions as indicated above. It was ripened to an acidity of 0.51 per cent (calculated as lactic acid), cooled to 7½° C. (45.5° F.), held overnight, during which the acidity rose to 0.65 per cent, and then churned. The buttermilk was drawn off and the butter allowed to remain unwashed, so as to contain the greatest amount of nonfatty ingredients of all three samples.

Since it was desired to have the three foregoing samples differ from one another only with respect to their buttermilk content, care was taken to prepare them otherwise in identically the same manner. Each was worked on a table worker to the extent of 40 revolutions, to incorporate a large quantity of air. They were then packed in clean and sterile glass jars, and also in the special glass tubes for air analysis. The butter in the jars was covered with a thin layer of paraffin to exclude any action of the atmosphere other than that confined within the material itself.

The appearance of undesirable flavors in stored butter has often been attributed to the use of either impure salt or water, or both, so this contingency was avoided by the use, in all cases, of chemically pure sodium chlorid and distilled water.

The samples were shipped by express to Washington, D. C., where they were kept in cold storage at a temperature of 0° F. Samples taken from the various lots packed in the jars were at once analyzed, and, in addition, were scored by Messrs. Corneliuson and Rabild, of the Dairy Division. After intervals of approximately one month, samples were withdrawn from storage, analyzed, and scored. This was continued for several months, during which time a sufficient period had elapsed for the samples to manifest any change which might occur in butter stored for a reasonable length of time. Of the three samples, designated for convenience as "excessively washed butter," "normally washed butter," and "unwashed butter," the first two will be given and discussed in conjunction (Tables VIII and IX).

TABLE VIII.—*Scores of excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.*

[Protein, 0.50 per cent. Total N×6.38]

| Age. | Score. | Remarks. | Scorer. |
|---|---|---|---|
| *Months.* | | | |
| 1 | 90 | Good, but trifle stale | Corneliuson. |
| 3 | 87 | .....do | Do. |
| 5 | 88 | .....do | Do. |
| 6 | 87 | .....do | Do. |

TABLE IX.—*Scores of normally washed butter, with normal content of nonfatty ingredients, stored at 0° F.*

[Protein, 0.57 per cent. Total N×6.38]

| Age. | Score. | Remarks. | Scorer. |
|---|---|---|---|
| *Months.* | | | |
| 1 | 91 | Flavor good | Corneliuson. |
| 2 | 89 | Trifle stale | Do. |
| 3 | 87 | Aroma good, trifle stale | Rabild. |
| 5 | 88 | Flavor good | Corneliuson. |
| 6 | 88 | .....do | Do. |

The keeping qualities of the two foregoing samples were practically the same, as shown by the scoring. The determination of the chemical constants gave the data in Tables X and XI.

TABLE X.—*Chemical constants of the fat of excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.*

[Protein, 0.50 per cent. Total. N×6.38]

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| Initial | 30.03 | 37.30 | 226.8 | 5.552 | 87.54 | 3.703 | 0.456 |
| 2 months | 29.83 | 36.52 | 226.4 | 5.623 | 87.63 | 3.578 | .458 |
| 4 months | 29.89 | 36.42 | 225.9 | 5.130 | 87.58 | 3.535 | .413 |

TABLE XI.—*Chemical constants of the fat of normally washed butter, with medium content of nonfatty ingredients, stored at 0° F.*

[Protein, 0.57 per cent. Total, N×6.38]

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| Initial | 30.03 | 37.30 | 226.8 | 5.552 | 87.54 | 3.703 | 0.456 |
| 2 months | 30.64 | 36.39 | 226.5 | 6.072 | 87.93 | 3.581 | .425 |
| 3 months | 30.16 | 36.98 | 226.1 | 5.490 | 87.56 | 3.942 | .459 |
| 5 months | 29.78 | 36.52 | 226.6 | 5.150 | 87.44 | 3.323 | .412 |

There is practically no variation in these figures from those obtained in the foregoing determination of chemical constants with the nearly pure butter fat standard. Evidently the fat in these two samples of butter underwent little or no chemical change, owing to the presence of either the confined air or the other nonfatty components. The analysis of this confined air, however, gave figures which differed considerably from those obtained in the analysis of the air confined within the samples of the butter fat itself (Table XII).

TABLE XII.—*Analysis of air in excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.*

[Calculated to 0° C. and 760 mm. Protein, 0.50 per cent. Total, N×6.38]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| *Months.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 2 | 31.88 | 4.48 | 14.05 | 5.52 | 17.31 | 5.50 |
| 3 | 42.50 | 2.77 | 6.52 | 6.47 | 15.23 | 7.95 |
| 4 | 26.57 | 2.01 | 7.57 | 3.39 | 12.76 | 4.91 |
| 5 | 26.28 | 1.94 | 7.38 | 3.24 | 12.33 | 4.87 |
| 14 | 30.55 | 1.74 | 5.70 | 1.84 | 6.03 | 5.78 |

[1] After deducting figure for carbon dioxid from total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

TABLE XIII.—*Analysis of air in normally washed butter, with medium content of nonfatty ingredients, stored at 0° F.*

[Calculated to 0° C. and 760 mm. Protein, 0.57 per cent. Total, N×6.38]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| *Months.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 2 | 27.88 | 1.85 | 6.64 | 5.26 | 18.87 | 5.21 |
| 3 | 29.99 | 3.97 | 13.24 | 4.34 | 14.47 | 5.20 |
| 4 | 26.26 | 3.45 | 13.14 | 3.72 | 14.17 | 4.56 |
| 14 | 28.94 | 3.90 | 13.47 | 2.41 | 8.33 | 5.01 |

[1] After deducting figure for carbon dioxid from total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

There is no great difference in the total quantity of carbon dioxid to be observed between these samples of excessively washed butter and what is considered to represent normally washed butter. It is to be noted that there is very little difference in the protein content of these two

samples. The high point (14.05 per cent) for carbon dioxid reached in the first case is about the same as that reached in the second (13.47 per cent), while there is no wide variation in the oxygen content of the two samples. The great decrease in the percentage of carbon dioxid in the former case occurs in the interval between the second and third months, after which this decreased percentage remains fairly constant. This decrease in the original percentage of carbon dioxid is also accompanied with a pronounced decrease in the percentage of oxygen. In the latter case the percentage of carbon dioxid increases to its maximum after the sample has been three months in storage, after which it remains fairly constant. It is to be noted, however, that the total amount of oxygen originally present in these samples of butter containing a certain proportion of buttermilk undergoes a markedly progressive decrease during the interval that the butter is kept in storage at a temperature of 0° F.

A survey of the data obtained from the sample of unwashed butter is of additional interest in this connection (Table XIV).

TABLE XIV.—*Scores of unwashed butter, with high content of nonfatty ingredients stored at 0° F.*

[Protein 0.90 per cent. Total N×6.38]

| Age. | Score. | Remarks. | Scorer. |
|---|---|---|---|
| *Months.* | | | |
| 1 | 92 | Oily, mottled | Corneliuson. |
| 2 | 89 | Oily, unclean | Rabild. |
| 3 | 87 | ....do | Corneliuson. |
| 5 | 85 | Slightly fishy | Do. |
| 6 | 86 | Stale, fishy, sour | Do. |

The progressive development of "off flavor" in this sample of butter, so prepared as to contain a greater quantity of buttermilk than either of the two foregoing samples, was remarkable. Since this butter had been prepared from a different lot of cream, it was necessary to establish a new fat standard of constants. The butter fat for this purpose was prepared from the same lot of cream as was the butter, and it was packed and stored in the same manner as that given for the previously mentioned sample of butter fat. The results are given in Tables XV and XVI.

TABLE XV.—*Chemical constants of butter fat stored at 0° F after being nearly freed from the nonfatty ingredients by melting, filtering, and washing*

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| Initial | 26.16 | 41.91 | 226.3 | 5.275 | 86.93 | 3.365 | 0.210 |
| 2 months | 26.90 | 41.40 | 225.9 | 5.147 | 87.46 | 3.579 | .220 |
| 3 months | 26.71 | 40.76 | 226.2 | 5.263 | 87.65 | 3.260 | .222 |
| 5 months | 26.93 | 40.79 | 226.5 | 5.220 | 87.55 | 3.335 | .214 |
| 6 months | 26.84 | 40.88 | 225.1 | 5.166 | 87.38 | 3.397 | .225 |

TABLE XVI.—*Chemical constants of the fat of unwashed butter, with high content of nonfatty ingredients, stored at 0° F.*

[Protein 0.90 per cent. Total N×6.38]

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| *Months.* | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| 1 | 26.28 | 41.80 | 226.6 | 5.29 | 87.1 | 3.90 | 0.205 |
| 2 | 26.76 | 40.40 | 226.4 | 5.06 | 86.66 | 3.795 | .210 |
| 3 | 26.90 | 40.81 | 226.3 | 5.32 | 87.02 | 3.381 | .226 |
| 5 | 26.83 | 40.13 | 226.5 | 5.27 | 87.44 | 3.331 | .231 |
| 6 | 26.84 | 40.30 | 225.0 | 5.17 | 86.98 | 3.294 | .226 |

The same comments are here to be made as in the case of the previous sample of nearly pure butter fat. No noteworthy chemical change had occurred in this sample of butter fat after having been kept in storage at a temperature of 0° F. for a period of six months. With respect to the fat taken from the sample of butter so prepared as to contain the greatest number of constituents in addition to the fat, the same observations are here to be made as in the previous cases of two different lots of butter containing smaller numbers of nonfatty constituents. The chemical constants here show little or no variation from those obtained with the nearly pure butter fat, and there is apparently no chemical change in the fat of butter prepared with a still greater number of substances in addition to the fat, owing either to the presence of these substances or to the presence of the confined air. An analysis of this confined air, however, gives some very striking data (Table XVII) when compared with those obtained in the foregoing samples.

TABLE XVII.—*Analysis of air in unwashed butter, with high content of nonfatty ingredients, stored at 0° F.*

[Calculated to 0° C. and 760 mm. Protein 0.90 per cent. Total N×6.38]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| *Months.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 2 | 29.26 | 9.19 | 31.41 | 0.71 | 2.43 | 4.01 |
| 3 | 28.23 | 8.94 | 31.67 | .62 | 2.20 | 3.86 |
| 4 | 29.15 | 8.13 | 27.89 | .47 | 1.61 | 4.20 |
| 5 | 38.19 | 9.97 | 26.12 | .37 | .97 | 5.64 |
| 15 | 33.28 | 7.41 | 22.26 | .37 | 1.11 | 5.17 |

[1] After deducting the figure for carbon dioxid from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

The maximum content of carbon dioxid (31.67 per cent) in this sample of unwashed butter was noticed after a storage period of three months, at about which time the characteristic "off flavor" became distinctly noticeable. At the end of two months there was very little oxygen in the sample; yet even this shows a perceptible decrease during the storage interval.

It has been indicated in the foregoing experiments that the quantity of carbon dioxid occurring in the gas inclosed in a package of stored butter is proportional to the amount of nonfatty ingredients incorporated into the material. There is also a more or less pronounced decrease in the oxygen content during the storage period. The following additional experiment was made with the view of confirming this relative change in the percentages of carbon dioxid and oxygen, and especially to determine the quantities of these gases occurring in unwashed butter at the time of its manufacture, since all the above-described analyses were made upon the samples after an interval of two months in storage.

For this purpose some unwashed butter was prepared from cream pasteurized at 145° F. for 20 minutes, and, as in the other cases, ripened with a pure culture. The cream was ripened to an acidity of 0.45 per cent (calculated as lactic acid), cooled to 7° C. (44.6° F.), held overnight, during which the acidity rose to 0.67 per cent, and then churned. The buttermilk was drawn off and the butter allowed to remain unwashed. The butter was then salted with chemically pure salt and worked on a table worker. This butter contained 4.72 per cent of sodium chlorid and 0.56 per cent of protein (total N×6.38). The butter was then packed into the special glass tubes for air analysis.

The gas in the first sample was extracted therefrom and analyzed as soon as possible after the butter was made—that is, 1½ hours. The remaining samples were kept at room temperature, but in the dark (Table XVIII).

TABLE XVIII.—*Analysis of air from a second sample of unwashed butter kept at room temperature but in the dark*

[Calculated to 0° C. and 760 mm. Protein 0.56 per cent. Total N×6.38]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 1½ hours | 37.7 | 7.5 | 19.89 | 7.7 | 20.42 | 6.0 |
| 2 days | 33.4 | 7.4 | 22.16 | 5.6 | 16.77 | 5.2 |
| 7 days | 33.8 | 7.5 | 22.19 | 5.1 | 15.09 | 5.3 |
| 14 days | 35.8 | 8.2 | 22.91 | 3.8 | 10.61 | 5.5 |

[1] After deducting the figure for carbon dioxid from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

Most of the carbon dioxid appears to have existed in the butter as soon as the manufacture of the material was completed. It also appears to increase somewhat in quantity during a period of two weeks. The oxygen figures show in a striking manner the decrease in the initial quantity of this gas present in the butter, and it is apparent that it has decreased to practically one-half this quantity after being kept two weeks at room temperature in the dark.

The question now arises whether there exists in the samples of butter fat the same homogeneous distribution of air bubbles as in the case of those samples of butter containing the varying quantities of nonfatty ingredients, for it is conceivable that the air incorporated into the butter fat may occur mostly in large pockets, while the other samples may contain, in addition, a certain amount of the total air inclosed within the particles of curd, lactose, etc.

In the first case it is reasonable to suppose that a smaller surface of material would be exposed to the influence of the air than in the second; yet it is improbable that this would alter the basic facts, since the analytical data obtained in the experiments indicate that the particles of nonfatty ingredients inclosing the air are more readily attacked by the oxygen therein than the fat itself. However, to obtain further confirmatory data on this point—that is, that the nonfatty constituents of butter are more readily attacked by the oxygen of the air incorporated into the material than the fat itself—the following experiments were conducted.

### BUTTERMILK EXPOSED TO A LARGE SURFACE OF AIR

Several of the special butter tubes were filled with large fragments of cracked and ignited pumice. The pumice of one lot of tubes was impregnated with the buttermilk from butter made from pasteurized cream acidified to 1 per cent with lactic acid before churning. The pumice of each tube of a second lot was treated with 10 c. c. of a 1 per cent solution of lactic acid. The tubes of these two lots were kept at a temperature of 32° F. At various times tubes from each were removed from storage and an analysis of the air in them was made. The analytical data obtained are given in Table XIX.

TABLE XIX.—*Oxidation of acid-cream buttermilk and of lactic acid exposed to the action of a large surface of air at a temperature of 32° F.*

| Acid buttermilk. | | | Lactic acid. | | |
|---|---|---|---|---|---|
| Period at 32° F. | Oxygen. | Carbon dioxid. | Period at 32° F. | Oxygen. | Carbon dioxid. |
| *Days.* | *Per cent.* | *Per cent.* | *Days.* | *Per cent.* | *Per cent.* |
| 4½ | 17. 67 | 2. 37 | 30 | 20. 70 | 0 |
| 26 | 0 | 34. 37 | 60 | 21. 07 | 0 |
| 62 | 0 | 31. 76 | 98 | 21. 01 | 0 |

The change in the composition of the air in contact with the acid buttermilk was very marked during a storage interval of only 26 days when this sample was kept at a temperature of 32° F. From a total percentage of 17.67 found to be present in the acid buttermilk when the material was 4½ days old, the oxygen content fell to zero during the period between this time and 26 days. The carbon-dioxid content of the buttermilk, initially small in quantity, rapidly increases to a maxi-

mum, from which point it begins to decrease. It is also very clear from the control experiment given in Table XIX that the change in the composition of the air inclosed in the material is not caused by decomposition of the lactic acid itself or to any action of this acid upon the particles of pumice.

It has already been shown in this paper that the acidity of the cream from which the butter is made has a direct influence on the change, during storage at a temperature of 0° F., in the composition of the air incorporated into the butter at the time of its manufacture. It has also been shown that but a slight change is to be observed in the composition of the air from a tube containing a small quantity of pure butter fat exposed to the action of a large and confined surface of air while the fat was kept at a temperature of 32° F. It has likewise been demonstrated that practically no change in the composition of the air occurs when pure butter fat is exposed to the action of about the same amount of air as is usually present in normal butter while it is stored at a temperature of 0° F. As it has been proved by an analysis of the air from a sample of sweet-cream butter made from cream of low acidity that this kind of butter suffers very little, if any, measurable decomposition during a period of six months in storage at a temperature of 0° F., and having also proved by other experiments that a decomposition of the fat of whole butter stored at a temperature of 0° F. for the same length of time is practically excluded, it is a logical conclusion that the particles of buttermilk inclosed in a sample of sweet-cream butter made from cream of low acidity likewise suffers little, if any, measurable decomposition when the butter is stored at a temperature of 0° F. It was decided, however, to settle this point definitely by experiment.

For this purpose the pumice of a third lot of tubes was impregnated with buttermilk from sweet-cream butter made from pasteurized cream having an acidity of 0.108 per cent (calculated as lactic acid). The acidity of the cream in this case was practically the same as that of the cream from which the foregoing sample of sweet-cream butter was made. This last lot of tubes containing the sweet-cream buttermilk was kept at a temperature of 0° F. (Table XX).

TABLE XX.—*Oxidation of sweet-cream buttermilk exposed to the action of a large surface of air at 0° F.*

| Period at 0° F. | Oxygen. | Carbon dioxid. |
|---|---|---|
| *Days.* | *Per cent.* | *Per cent.* |
| 35 | 20.92 | 0 |
| 65 | 20.93 | 0 |
| 270 | 20.25 | 0 |

That the sweet-cream buttermilk underwent practically no change in storage at a temperature of 0° F. is shown by the foregoing data.

## SUMMARY AND CONCLUSIONS

The composition of the air confined within a package of pasteurized sweet-cream butter known to contain bacteria and made from cream having an acidity of 0.11 per cent (calculated as lactic acid) showed little or no variation from its original composition after successive periods in storage, aggregating six months, at a temperature of 0° F. A small quantity of the buttermilk from butter made from pasteurized sweet cream having the same low degree of acidity as the cream above mentioned, when exposed to the influence of a very large and confined surface of air, appeared to have little, if any, effect upon the original composition of the air when the buttermilk was stored for nine months under like conditions of temperature. A portion of this same sample of sweet-cream butter when kept at a temperature of 32° F. showed a decided change in the original composition of the inclosed air, a change which was still further increased when the butter remained for a short time at room temperature. This change in the composition of the air originally incorporated into the butter was expressed by a decrease in the percentage of oxygen and a corresponding increase in the percentage of carbon dioxid. This sample of sweet-cream butter still possessed a good score after six months' storage at a temperature of 0° F., there being no indication of any undesirable flavor.

The change in the composition of the air initially inclosed within a package of butter made from sweet cream and churned immediately after the addition of 15 per cent of a commercial starter showed but little variation from that observed in the sample of sweet-cream butter when the two samples were kept under comparable conditions, both being in storage at a temperature of 0° F., although the acidity of the cream in the first case was somewhat higher (0.25 per cent) than that of the cream from which the sweet-cream butter was made. This sample of butter also displayed good keeping qualities during its storage period of nearly seven months at a temperature of 0° F.

The composition of the air inclosed within a package of butter made from sweet cream and churned immediately after the addition of lactic acid, the total acidity of the cream being about six and one-half times greater than that of the cream from which the sweet-cream butter was made, showed pronounced variations from its original composition during successive periods of storage at a temperature of 0° F. These variations were still greater when the sample was allowed to stand at a temperature of 32° F. In this case there was a considerable and a progressive decrease in the original oxygen content, as well as in the original carbon-dioxid content. A small quantity of the buttermilk from butter made from pasteurized sweet cream and churned immediately after the addition of lactic acid, when exposed to the action of a very large and confined surface of air under the same temperature conditions, showed precisely the

same phenomena with respect to alteration in the original air composition. The oxygen content of the confined air had entirely disappeared within a month's time. The carbon-dioxid content, originally 2.37 per cent, had increased to more than 34 per cent within the same interval, after which time it had begun to decrease. The flavor of this butter, which was prepared from pasteurized sweet cream and churned immediately after the addition of lactic acid, was somewhat unclean after a storage period of only three months at a temperature of 0° F., and decidedly so after being in storage for six months under the same conditions.

Further, it has been indicated by the investigation pursued with pasteurized, ripened-cream butter through the successive steps from nearly pure butter fat to samples of butter containing varying quantities of ingredients other than fat and, finally, to samples containing the greatest quantity of protein, lactose, etc., and stored for a reasonable length of time (six months) at a temperature of 0° F., that the amount of carbon dioxid inclosed in a package of the material is directly proportional to the quantity of these ingredients contained therein. It has been shown that this quantity of carbon dioxid may increase during the earlier part of the storage period, followed by a decrease during the latter part. It is also of especial significance, perhaps, that the oxygen content of the gas in the material undergoes a marked and striking decrease during the interval that the samples of butter containing the varying amounts of constituents other than fat are retained in storage, and that this decrease is likewise proportional to the amount of acid and ingredients other than fat contained in the butter.

The fat of butter made from pasteurized cream, on the contrary, undergoes no apparent oxidation during the same storage period when kept at a temperature of 0° F. It is only when a substance like pumice, which may have catalytic properties, is impregnated with a small amount of butter fat and exposed to the action of a large amount of air while kept at a temperature of 32° F. that a very slight oxidation is noticeable.

The results of the investigations may be summed up as follows:

(1) The development of undesirable flavors in butter held in cold storage at a temperature of 0° F. is not dependent upon an oxidation of the fat itself.

(2) The production of "off flavors" so commonly met with in cold-storage butter is attributable to a chemical change expressed through a slow oxidation progressing in some one or more of the nonfatty substances occurring in the buttermilk.

(3) The extent of this chemical change is directly proportional to the quantity of acid present in the cream from which the butter was prepared.

(4) The quantity of carbon dioxid present in cold-storage butter appears to have a certain relation to the quantity of buttermilk in the butter. During storage this quantity of carbon dioxid may increase to a maximum followed by a progressive decrease.

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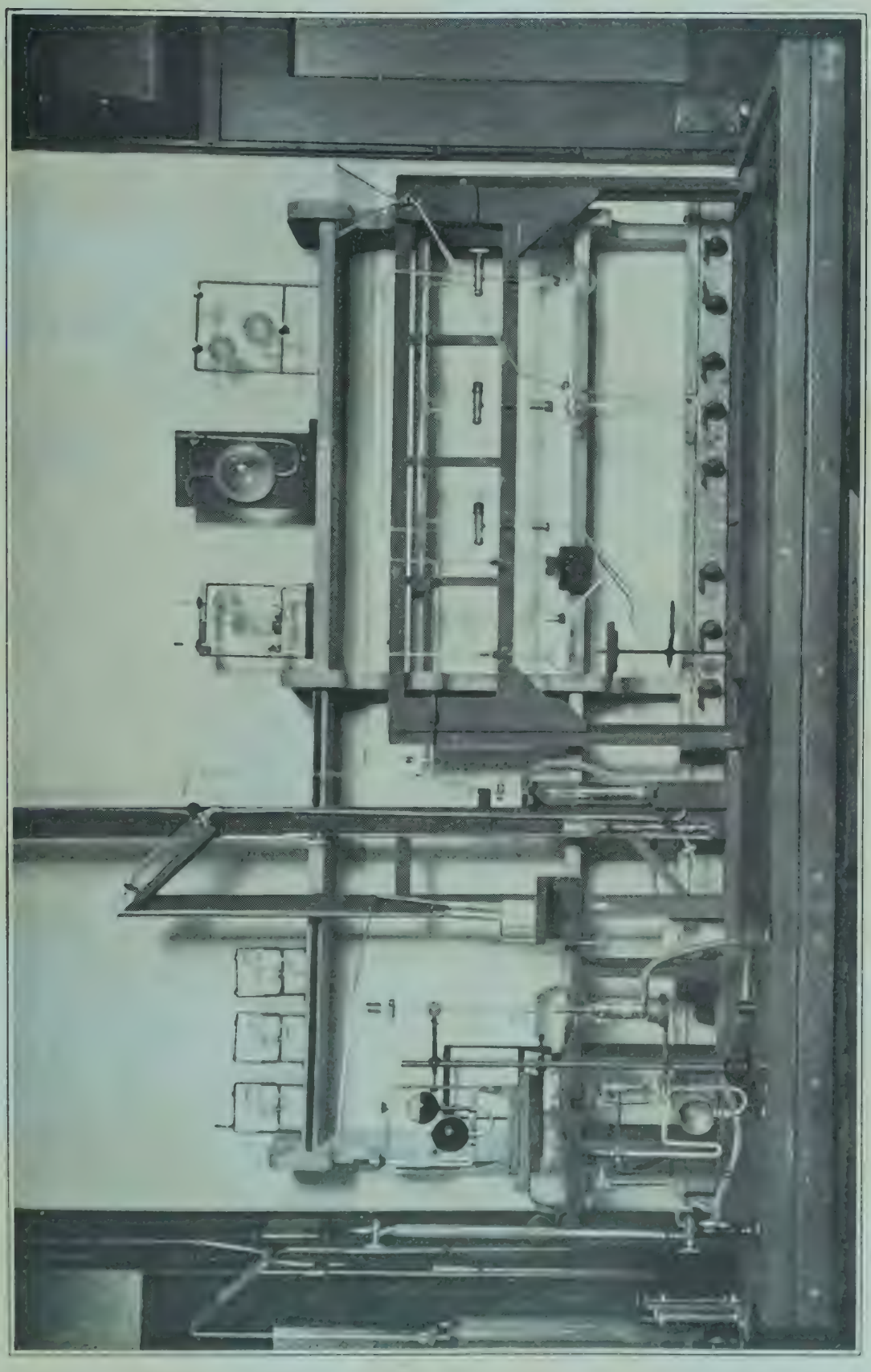
## PLATE CXI

Gas apparatus used in the extraction and analysis of the air confined in butter.



# BACTERIOLOGICAL STUDIES OF A SOIL SUBJECTED TO DIFFERENT SYSTEMS OF CROPPING FOR TWENTY-FIVE YEARS

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## INTRODUCTION

During the past few years a number of soil biologists have reported their findings regarding the effects of different agricultural practices upon the bacteria of the soil. The majority of these investigations have been concerned with determining the gross effects of a particular treatment upon the physiological activities of the flora as a whole. Such factors as continuous cropping, rotational systems, cultural methods, application of chemical fertilizers, and manures have been studied. Among the more recent workers reporting such investigations in the United States may be mentioned King and Doryland (10),[1] Stevens and Withers (18), Stewart and Greaves (19), Lyon and Bizzell (12), Temple (20), Jensen (9), Given and Willis (6, 7), Brown (2, 3, 4), Hill (8), Allen and Bonazzi (1), Wright (22), and McBeth and Smith (13).

It is not necessary to give any extensive review of the work that has been done as the papers referred to above contain full summaries of the results thus far obtained. Special attention may be called to the review given by Temple (20) as to the effect of stable manure, by Hill (8) as to the effect of other organic materials, and by Lyon and Bizzell (12) as to the effect of different growing crops. The available data leave little doubt that certain of the above-mentioned factors do exert a marked effect upon soil organisms. In very few instances, however, has any serious effort been made to ascertain just how such factors exert their influence. In most instances the treatment in question has been in operation a comparatively short time. Brown (3), for example, studied the effects of a 4-year rotation while the fourth crop was still on the soil, or before the cycle was completed the first time. It is true that the quantities of nitrate nitrogen have been determined *in situ*, following long-continued cropping systems. With our present very limited knowledge as to the demands any particular crop makes upon soil nitrates, such information gives us little insight into nitrate formation. Allen and Bonazzi (1) carried out a few laboratory experiments, following a long-continued treatment, but obtained such irregular results that they regarded them of little value. Given and Willis (6, 7) have also re-

[1] Reference is made by number to "Literature cited," p. 974–975.

ported a limited number of experiments following 30 years of continuous treatment.

It was with the ultimate object of attempting a study of the fundamental causes for variations in certain changes in soil nitrogen that this work was undertaken. Before it could be begun, however, it was first necessary to determine the existence of differences under our conditions. Furthermore, it seemed desirable to compare the relative effect of long-continued treatment with that of the shorter periods reported by others.

The Missouri Experiment Station possesses a series of fertility plots that offered exceptional opportunity for making the above-mentioned studies. The plots cover a rather wide range and have just passed their twenty-fifth year of continuous treatment as outlined in the original project. Some very valuable data as to the effect upon fertility as measured by crop-producing power have been obtained. It would seem as if 25 years would have so materially changed the micro-organic life therein that such could readily be detected, provided such differences were actually brought about. With such material upon which to work it would seem that the verity of marked differences in similarly treated soil reported by others could be established.

The data herein reported have been obtained from some of the plots that have given the most marked differences in yield, as it was believed such plots would offer the best material upon which to work. The work, however, is concerned only with demonstrating the existence of differences and offers only one or two suggestions as to the actual cause of such differences. We hope to be able to throw more light upon this particular field at a later date. Furthermore, the particular data here reported have to do only with bacterial numbers and with ammonia and nitrate-forming abilities.

Certain facts, which, we believe, we have very clearly demonstrated, have been of value to us in directing further work. It is with the hope that the data may be of similar value to others that we present them in this paper.

## PLOTS STUDIED

The fertility plots of the Missouri Experiment Station are located on the soil type classed as Putnam silt loam. They were first planted to the present system in 1889 and, with few irregularities, have received the same treatment as outlined. Each plot consists of one-tenth acre and is surrounded by an alley 3 feet wide. In selecting from the large number of plots the few that could be handled in our work, an effort was made to include as wide a variation of treatment as possible and at the same time to avoid inherent soil differences in order that the work might be comparative.

The plots studied are, with the exception of No. 29 and 30, located just at the crest and on the eastern slope of a gentle rise. Plots 29 and

30 are of a slightly different texture, being located on the western slope of the same rise. For this reason they are omitted from the general scheme and are compared with each other only. The greatest distance between any two plots (No. 1 and 23) is only about 85 yards. It was impossible to get the specially treated plots that we wished to study located any closer together. It is not believed, however, that any difference due to character of soil, location, etc., could materially affect the major differences reported. The plots studied, treatment received, and yields are given in Table I. It will be noted that the plots studied during 1914 varied somewhat from those of the previous year. This was necessary because of a change in treatment in certain plots beginning in 1914. In the tabulated data, plot 1 always includes the data obtained from plot 20 (a duplicate). Similarly plot 10 includes data secured from plot 21 (a duplicate).

Where stable manure has been applied, it has been an annual application averaging 6.7 tons per acre on all plots except No. 1, which received 7 tons. The chemicals were applied annually on plots 2 and 3 in the form of sodium nitrate, potassium chlorid, and acid phosphate in quantities sufficient to supply nitrogen, potassium, and phosphorus for a full yield of the particular crop. In the case of wheat this was for a yield of 40 bushels. The rotation consisted of corn, oats, wheat, clover, timothy, and timothy. The other plots have been annually planted to the specific crop mentioned.

TABLE I.—*Cropping system, treatment, and yields of the various fertility plots studied at Columbia, Mo.*

| Plot No. | Cropping system. | Treatment. | Year studied. | Yield. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1914 | 1913 | 1909–1913 | 1904–1913 | 1889–1913 |
| 1 | 6-year rotation [a] | Stable manure | 1914 | 1,326 pounds | 3,887 pounds | | | |
| 2 | Continuous wheat | Chemicals | 1914 | 20.32 bushels | 17.01 bushels | 12.83 bushels | 16.38 bushels | 17.99 bushels. |
| 3 | 6-year rotation | do | 1914 | 964 pounds | 2,964 pounds | | | |
| 9 | Continuous wheat | None | 1914 | 20.09 bushels | 8.83 bushels | 6.41 bushels | 6.54 bushels | 9.56 bushels. |
| 10 | do | Stable manure | 1914 | 26.37 bushels | 16.90 bushels | 12.21 bushels | 12.47 bushels | 16.72 bushels. |
| 13 | 6-year rotation | None | 1913–14 | 377 pounds | 1,508 pounds | | | |
| 17 | Continuous corn | do | 1913–14 | 28.78 bushels | 6.96 bushels | 10.08 bushels | 10.68 bushels | 20.35 bushels. |
| 18 | do | Stable manure | 1913–14 | 33.89 bushels | 19.22 bushels | 19.64 bushels | 24.54 bushels | 35.78 bushels. |
| 20 | 6-year rotation | do | 1913 | 1,177 pounds | 4,810 pounds | | | |
| 21 | Continuous wheat | do | 1913 | 28.41 bushels | 17.44 bushels | 14.35 bushels | 15.82 bushels | 18.66 bushels. |
| 22 | Continuous timothy | do | 1913–14 | 1,482 pounds | 3,042 pounds | 4,388 pounds | 4,345 pounds | 5,006 pounds. |
| 23 | do | None | 1913–14 | 910 pounds | 1,339 pounds | 2,075 pounds | 2,264 pounds | 2,853 pounds. |
| 29 | Continuous wheat | Stable manure [b] | 1913 | 29.56 bushels | 16.79 bushels | 11.87 bushels | 12.59 bushels | 15.61 bushels. |
| 30 | do | do | 1913 | 28.17 bushels | 22.53 bushels | 12.66 bushels | 14.96 bushels | 19.58 bushels. |

[a] The 6-year rotation plots were in clover during 1913 and timothy during 1914.

[b] Stable manure since 1908.

## EXPERIMENTAL METHODS EMPLOYED

Practically all the methods here mentioned have been severely criticised during the past few years, particularly by Löhnis and Green (11), Allen and Bonazzi (1), and Noyes (14). However, in our laboratory the methods described below have proved, for the object in view, equal or superior to any suggested prior to the beginning of this work.

Samples for the various analyses were taken with a 1½-inch soil auger. Ten to fifteen samples were collected from each plot to the depth of the soil, which was about 10 inches. The cores of soil were taken uniformly all over the plot, avoiding close proximity to the surrounding alleys. They were placed immediately in sterile Mason jars in order to prevent loss of moisture and to avoid contamination, so far as possible. The samples were then brought to the laboratory as soon as possible, where, under aseptic conditions, the soil was passed through a 2-mm. sieve and thoroughly mixed. Samples were immediately taken for quantitative analyses and for moisture determinations.

For moisture determinations 50 gm. of soil were dried at 110° C. for two hours. To determine the water-holding capacity, 50 gm. were placed in a carbon filter containing a perforated porcelain bottom and a measured quantity of water poured on top. The water was permitted to percolate through the soil. The process was repeated two or three times. From the amount of water absorbed plus the quantity lost in drying, the water-holding capacity, expressed in grams of water held per 100 gm. of dry soil, was determined. Data obtained by these methods are, of course, not absolute. But the process possesses two essentials: Quickness of manipulation and comparativeness. Since slight differences in water content, when near the optimum, exercise but little influence upon bacterial activity, it is believed the error introduced is not appreciable.

Quantitative analyses were made by carefully weighing 1 or 2 gm. of soil, placing it in 98 or 99 c. c. of sterile water and shaking vigorously for one minute. From this suspension dilutions were made in the ordinary way. Finally, 1 c. c. was placed in each of three sterile Petri dishes and thoroughly mixed with 10 c. c. of Temple's agar (20). The dishes were then incubated for one week at room temperature and all colonies counted with the aid of a hand lens. If one of the dishes varied widely from the other two it was discarded. The same was true if dishes were overrun by spreaders or molds. The results are reported in millions of bacteria per gram of dry soil.

The ammonia- and nitrate-forming experiments were carried out by thoroughly mixing into fresh soil (the equivalent of 100 gm. of dry soil) sterile cottonseed meal containing 60 mgm. of nitrogen. This was placed in a sterile 500 c. c. wide-mouthed bottle and the moisture con-

tent made up to the optimum (two-thirds water-holding capacity). Two samples were incubated for one week and two for four weeks. The ammonia and nitrate nitrogen were then determined and reported as milligrams of nitrogen and nitrates ($NO_3$), respectively, per 100 gm. of soil. In 1913 the ammonia was determined as follows: The water content was made up to a definite volume and the whole shaken for 45 minutes. Two gm. of calcium oxid were then added and the contents were again shaken for a short time and allowed to stand until the supernatant liquid became clear. A definite volume of this liquid was then distilled in the presence of magnesium oxid, the distillate being collected in standard acid and titrated. The calcium oxid was added as a clarifying agent in order to obtain a solution upon which nitrate and nitrite nitrogen could be determined colorimetrically. The presence of this reagent caused a perceptible increase in the ammonia set free, probably liberating some of the loosely attached nitrogen; but since the results are comparative and because of reasons already mentioned, the method seemed justifiable. However, such insignificant quantities of nitrate and nitrite nitrogen were found after seven days' incubation that these determinations were discontinued during 1914, and the ammonia was determined by direct distillation of the soil in copper flasks.

Where the incubation lasted for four weeks, the water loss by evaporation from the soil was replaced from time to time. Besides these experiments, samples were also run during 1914 with the addition of calcium carbonate in excess of that required to neutralize all nitric acid that could be formed from the cottonseed meal. Nitrate nitrogen was determined in all cases upon an aliquot part of a solution, obtained as directed above, using the phenoldisulphonic-acid colorimetric method.

The nitrifying inoculation experiments were conducted as follows: A soil possessing both a high nitrifying capacity and nitrifying efficiency, in the sense that Stevens and Withers (17) use these terms, was selected as a standard medium. To 100-gm. samples of this soil, cottonseed meal containing 60 mgm. of nitrogen was added, sufficient water added to bring it up to optimum, less 20 c. c., and the whole subjected to 20 pounds' pressure for one hour in the autoclave. These samples were then inoculated from the various plots with 20 c. c. of a soil suspension made by shaking 1 part of soil in 2 parts of water. The incubation covered a period of 28 days at room temperature. Ammonia and nitrates were determined as stated above. The results are reported in milligrams of nitrogen and nitrate ($NO_3$), respectively, per 100 gm. of soil. It will be noted that the nitrate data for 1913 are low, in many places zero. This was caused by the failure to add calcium carbonate which was added in 1914. Apparently some substance toxic to nitrification is produced by heating. This substance gradually disappears on standing, and the disappearance is materially hastened by the addition of calcium carbonate.

The cross-inoculation experiments were tested by taking a mixture of all samples collected during both seasons from the respective plots, thoroughly mixing, and using as a medium 100-gm. samples containing 60 mgm. of nitrogen. These samples were also subjected to 20 pounds' pressure for one hour in the autoclave. A sufficient number of samples were thus prepared for duplicate inoculation from each plot under study. One of the duplicates received calcium carbonate; the other did not. These samples were incubated for six instead of four weeks and nitrate nitrogen determined as before.

In all the above-outlined experiments duplicate samples were set up and analyzed. Where possible, as in the nitrate determinations, duplicate determinations were run with each sample. If these varied widely, they were again run; or where this was impossible, they were discarded. In general, the duplicates agreed very well, except in the nitrifying inoculating experiments when incubated for only four weeks with no calcium carbonate added. Perhaps these results should not be included in the tabulated data; but since the relative positions of the averages do not materially differ from those of 1914, they have been included.

## EXPERIMENTAL WORK

### NUMBER OF BACTERIA

Table II gives the moisture content of soil from the different plots at the various samplings.

TABLE II.—*Percentage of moisture in soil of the fertility plots when sampled* [a]

| Plot No. | 1913 | | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 15.5 | 5.0 | 6.0 | 20.0 | 21.3 | 13.56 | 7.6 | 7.6 | 10.6 | 20.8 | 19.5 | 13.20 |
| 13 | 16.0 | 4.0 | 4.8 | 18.0 | 20.9 | 12.75 | 8.0 | 6.5 | 7.6 | 21.0 | 20.6 | 12.74 |
| 3 | ..... | ..... | ..... | ..... | ..... | ..... | 7.0 | 6.0 | 9.0 | 20.8 | 20.0 | 12.56 |
| 10 | 18.0 | 14.2 | 12.0 | 17.5 | 24.3 | 17.20 | 10.0 | 9.0 | 17.0 | 22.2 | 20.0 | 15.64 |
| 9 | ..... | ..... | ..... | ..... | ..... | ..... | 8.0 | 7.4 | 12.0 | 21.6 | 18.8 | 13.56 |
| 2 | ..... | ..... | ..... | ..... | ..... | ..... | 8.0 | 8.1 | 12.0 | 20.0 | 17.8 | 13.20 |
| 18 | 15.0 | 7.6 | 7.0 | 19.5 | 20.4 | 13.0 | 14.0 | 14.7 | 10.0 | 21.0 | 20.0 | 15.94 |
| 17 | 16.0 | 6.8 | 7.4 | 18.4 | 21.0 | 13.92 | 13.0 | 14.7 | 8.6 | 17.1 | 18.0 | 14.28 |
| 22 | 16.0 | 5.8 | 6.2 | 17.4 | 21.2 | 13.32 | 8.4 | 9.4 | 15.0 | 22.2 | 22.8 | 15.56 |
| 23 | 16.0 | 6.2 | 7.0 | 19.0 | 21.5 | 13.90 | 11.2 | 7.4 | 14.8 | 20.4 | 21.6 | 15.08 |

[a] The soil was dried at 110° C. for two hours.

Table III gives the total number of bacterial colonies developing on Temple's agar expressed in millions per gram of dry soil. More emphasis should be placed upon the 1914 series than the 1913, because of the larger number of analyses and the greater uniformity of plots. A study of this table brings out several interesting facts, the most evident being that of the effect of manure. In all cases except the rotated plot for 1913, all those receiving manure rank materially higher than those not receiving it. Since this exception did not hold true for 1914, it is

possible that some other factor was influencing plot 20. The plots receiving chemical fertilizers ranked a little lower than the lowest receiving manure but materially higher than those receiving nothing.

TABLE III.—*Number of bacteria per gram of soil* [a]

[,000 omitted]

| Plot No. | 1913 | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | July 11. | Aug. 12. | Nov. 4. | Dec. 20. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 7,800 | 4,000 | ..... | 1,270 | 4,360 | 5,000 | 4,500 | 4,800 | 8,100 | 7,000 | 5,880 |
| 13 | 3,690 | 7,080 | 4,590 | 1,520 | 4,210 | 2,820 | 2,300 | 4,400 | 4,000 | 5,560 | 3,820 |
| 3 | ..... | ..... | ..... | ..... | ..... | 4,800 | 3,000 | 5,000 | 6,000 | 7,560 | 5,270 |
| 10 | 10,730 | 9,900 | ..... | 6,270 | 8,960 | 7,050 | 4,830 | 11,800 | 15,600 | 26,000 | 13,050 |
| 9 | ..... | ..... | ..... | ..... | ..... | 1,840 | 1,800 | 3,500 | 4,300 | 3,450 | 2,980 |
| 2 | ..... | ..... | ..... | ..... | ..... | 4,730 | 3,150 | 6,480 | 4,560 | 8,100 | 5,400 |
| 18 | 4,600 | 8,000 | 14,000 | 4,820 | 7,855 | 10,400 | 15,400 | 9,000 | 14,750 | 17,100 | 13,330 |
| 17 | 2,950 | 3,860 | 3,400 | 1,670 | 2,970 | 2,760 | 3,000 | 2,680 | 3,100 | 4,400 | 3,190 |
| 22 | 10,840 | 3,610 | 5,900 | 2,300 | 5,660 | 3,710 | 4,560 | 7,600 | 10,260 | 16,200 | 8,470 |
| 23 | 4,520 | 3,160 | 5,430 | 920 | 3,500 | 2,800 | 3,300 | 4,650 | 4,500 | 7,000 | 4,450 |

[a] The soil was dried at 110° C. for two hours.

If the effect of the various cropping systems in the absence of manure is considered, the various systems rank in the following order in 1914: (1) Timothy, (2) rotation, (3) corn, and (4) wheat. Wheat and corn are about equal, with a marked increase in the rotated and the timothy soils. In the presence of manure the rank is almost the reverse: (1) Corn, (2) wheat, (3) timothy, and (4) rotation. Here, again, wheat and corn are approximately equal, with a marked falling off to timothy and a somewhat less decrease to the rotation.

Just why manure should have the effect of raising the bacterial content of wheat and corn from the lowest to the first rank is not known. On the other hand, just why it should have less effect upon plots with a normally higher count is equally not understood. The peculiar behavior of the rotation plot receiving manure is very striking, the bacterial numbers being only slightly affected. Particularly is this so when we remember that this rotation is composed of corn, oats, wheat, clover, and timothy, since corn, wheat, and timothy bring about conditions which readily respond to manure. It should be remembered that if samples had been taken from rotation plots when some crop other than clover or timothy was growing, the results might have been different. A possible explanation of the lack of effect of manure upon the rotation plots and a less-marked effect upon timothy may lie in the amount of organic matter that these plots themselves return to the soil. The less the quantity of organic matter returned to the soil apparently the more marked is the result from manure.

The results secured from eight analyses of plots 29 and 30 (not given in Table III) indicate that the effect of manure is in part accumulative. Plot 29 had received only 6 applications of manure against 25 for plot 30,

while the average bacterial count for No. 29 was 7,840,000 and for No. 30 was 13,632,000. This accords with Temple's idea (20) that the increase in number is largely due to the fermentable material added and not to the bacteria actually carried in the manure.

### FORMATION OF AMMONIA

The results presented in Table IV seem to the writers clearly to establish one of two facts, either that the systems of cropping under study exert no appreciable influence on the ammonia-forming power of this soil type or that the methods used for determining such differences are valueless. Since many investigators have been able to detect marked differences with essentially the same methods, the former conclusion might seem most likely. We would call special attention, however, to the results obtained by Given and Willis (6, 7) and Perotti (15), together with their conclusions regarding the existence of differences and the value of methods in vogue for determining this phenomenon. It is true that during 1913 the plots receiving manure gave slightly higher results than those receiving no such treatment, but it is equally true that the reverse is evident for 1914. Furthermore, there seems to be no correlation between the number of bacteria and the amount of ammonia formed.

It may be argued, in view of the nitrification data given later, that in the plots receiving manure the ammonia formed was transformed into nitrates. But, as previously mentioned, the accumulation of nitrates in seven days' time in the presence of cottonseed meal is practically nil. In fact, the senior writer (5) has demonstrated that under such conditions there is a rapid disappearance of nitrate nitrogen during the first few days. To ascertain this condition, determinations of nitrate nitrogen were made during 1913 which showed, as may be noted, that the above contention was verified. The greatest quantity of nitrate nitrogen recovered after one week's incubation was 3.5 mgm. from plot 10. It is true that there were large differences in the amount of ammonia in the four-week analyses; but if we add the nitrate and ammonia nitrogen, the differences are slight. From the ammonia figures it is also evident that in no case, even after four weeks' incubation, in the absence of calcium carbonate was nitrification limited by the formation of ammonia. This possibly did not hold true in a few cases when calcium carbonate was added.

### FORMATION OF NITRATE

In our study of nitrate formation we have obtained the most marked and consistent results of any of our studies. This is graphically shown in figure 1 for the four-week incubated samples without calcium carbonate. Tables IV and V give the tabulated results of experiments conducted on nitrate formation in natural soil from different plots.

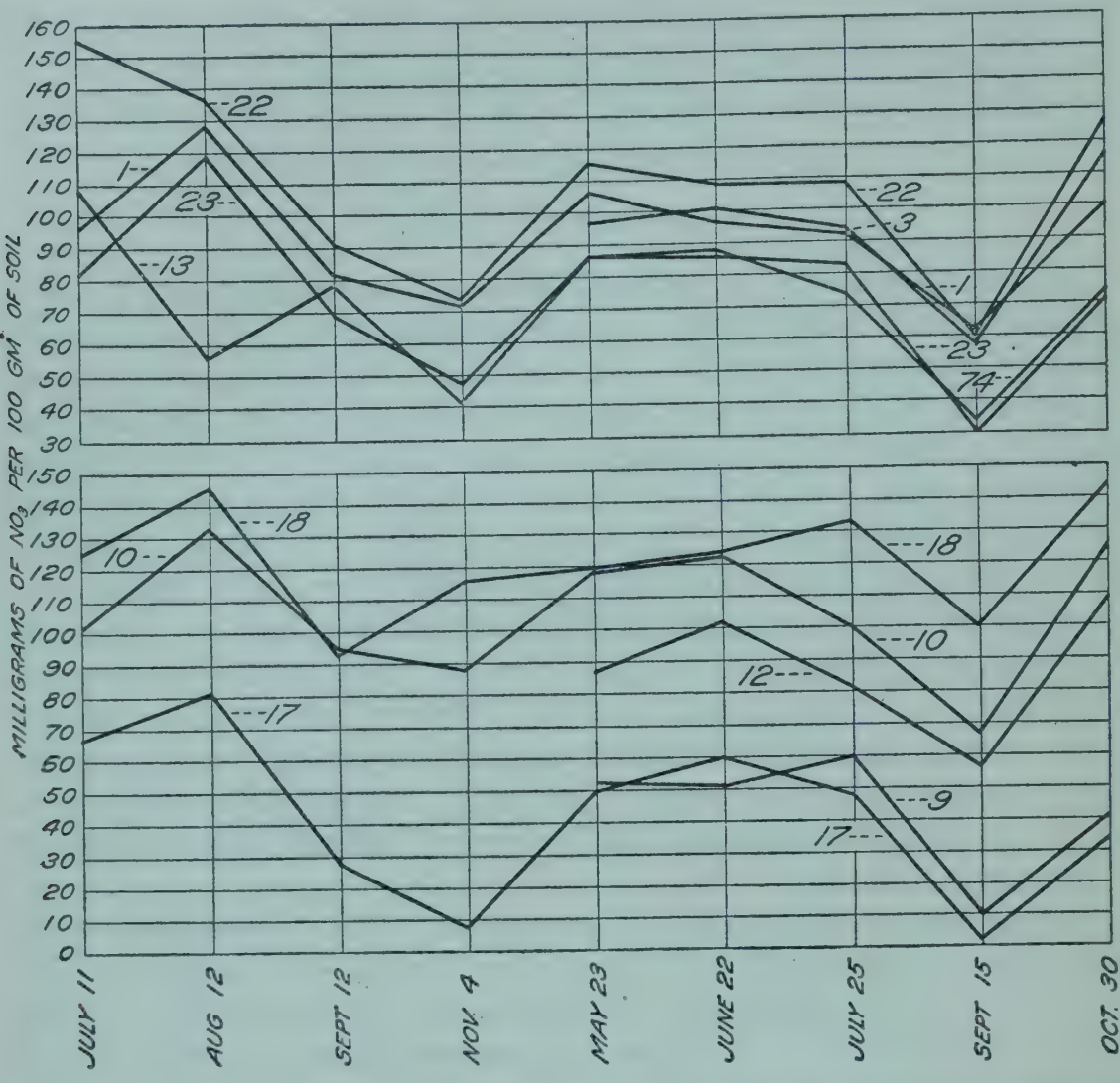


FIG. 1.—Curves of the nitrate formation in soil of fertility plots at Columbia, Mo., in 1913–14, after 28 days' incubation with the addition of 60 mgm. of nitrogen as cottonseed meal, but without the addition of calcium carbonate.

TABLE IV.—*Ammonia and nitrate formation (in milligrams) in soil from different fertility plots at Columbia, Mo., during 7 days' incubation without addition of calcium carbonate*

| Plot No. | 1913 | | | | | | | | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrate ($NO_3$) recovered. | | | | Nitrogen as ammonia recovered. | | | | Nitrogen as nitrate and ammonia recovered. | | | | Nitrogen as ammonia recovered. | | | | | |
| | Aug. 12. | Sept. 12. | Nov. 4. | Average. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1..... | 3.76 | 8.95 | T.[a] | 4.24 | 39.67 | 27.87 | 31.63 | 33.06 | 40.52 | 29.88 | 31.63 | 34.01 | 27.97 | 26.82 | 24.94 | 20.31 | 24.68 | 24.94 |
| 13..... | T.[a] | 2.43 | 0 | .80 | 40.35 | 29.14 | 32.70 | 34.06 | 40.35 | 29.69 | 32.70 | 34.24 | 28.31 | 29.71 | 26.88 | 24.00 | 26.99 | 27.18 |
| 3..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 28.43 | 27.93 | 25.79 | 20.50 | 25.41 | 25.61 |
| 10..... | 4.30 | 15.78 | T.[a] | 6.69 | 46.27 | 31.67 | 34.23 | 37.37 | 47.23 | 35.22 | 34.23 | 38.89 | 21.84 | 25.31 | 24.52 | 21.70 | 24.03 | 23.48 |
| 9..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 28.10 | 27.93 | 26.22 | 21.70 | 25.90 | 25.99 |
| 2..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 27.88 | 28.67 | 27.63 | 21.70 | 28.81 | 26.94 |
| 18..... | 6.69 | 5.09 | T.[a] | 3.93 | 37.20 | 31.25 | 36.53 | 34.99 | 38.70 | 32.40 | 36.53 | 35.87 | 21.65 | 24.40 | 24.52 | 19.64 | 24.44 | 22.93 |
| 17..... | 0 | 1.41 | 0 | .47 | 38.70 | 27.87 | 30.55 | 32.37 | 38.70 | 28.19 | 30.55 | 32.48 | 28.40 | 27.00 | 28.22 | 16.74 | 27.42 | 25.55 |
| 22..... | 3.78 | 6.38 | 0 | 3.39 | 42.52 | 25.76 | 31.85 | 33.38 | 43.32 | 27.19 | 31.85 | 34.14 | 26.62 | 28.67 | 25.85 | 23.39 | 25.83 | 26.07 |
| 23..... | 0 | 4.06 | 0 | 1.35 | 43.15 | 25.33 | 29.03 | 32.50 | 43.15 | 26.23 | 29.03 | 32.80 | 26.93 | 28.30 | 25.61 | 22.19 | 24.32 | 25.47 |

[a] T.=trace.

TABLE V.—*Ammonia and nitrate formation (in milligrams) in soil from different fertility plots at Columbia, Mo., during 28 days' incubation with and without addition of calcium carbonate*

| Plot No. | 1913 (without calcium carbonate). | | | | | | | | | | | | | | | 1914 (without calcium carbonate). | | | | | | 1914 (with calcium carbonate). | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrate ($NO_3$) recovered. | | | | | Nitrogen as ammonia recovered. | | | | | Nitrogen as nitrate and ammonia recovered. | | | | | Nitrate ($NO_3$) recovered. | | | | | | Nitrate ($NO_3$) recovered. | | | | | |
| | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 95.8 | 128.0 | 82.4 | 72.6 | 94.7 | 9.49 | 24.74 | 18.78 | 31.86 | 21.22 | 31.16 | 53.69 | 37.42 | 48.28 | 42.64 | 105.8 | 97.3 | 92.3 | 62.0 | 100.0 | 91.5 | 141.0 | 133.3 | 144.0 | 146.0 | 175.6 | 147.9 |
| 13 | 109.4 | 56.7 | 79.2 | 42.4 | 71.9 | 10.41 | 29.14 | 28.40 | 38.14 | 26.52 | 35.16 | 41.97 | 46.31 | 47.73 | 42.78 | 86.4 | 87.8 | 75.0 | 34.2 | 74.2 | 71.5 | 161.5 | 153.3 | 138.6 | 58.8 | 163.6 | 135.2 |
| 3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 97.3 | 100.7 | 94.7 | 58.0 | 116.1 | 93.4 | 150.0 | 156.5 | 138.6 | 92.3 | 165.5 | 140.6 |
| 10 | 101.1 | 133.0 | 95.6 | 88.3 | 104.5 | 8.35 | 22.01 | 19.41 | 22.77 | 18.14 | 31.22 | 51.79 | 41.03 | 42.74 | 41.69 | 120.0 | 124.1 | 100.0 | 67.9 | 124.1 | 107.2 | 164.4 | 147.0 | 150.0 | 120.0 | 165.6 | 149.4 |
| 9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 52.2 | 51.4 | 60.0 | 10.5 | 41.5 | 43.1 | 139.8 | 125.9 | 129.7 | 50.7 | 171.4 | 123.5 |
| 2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 87.8 | 102.8 | 81.8 | 56.3 | 109.0 | 87.5 | 144.6 | 153.2 | 144.0 | 73.4 | 158.3 | 134.7 |
| 18 | 125.2 | 146.3 | 93.2 | 116.2 | 120.2 | 6.14 | 6.92 | 17.52 | 21.02 | 12.90 | 34.46 | 39.99 | 38.60 | 47.30 | 40.09 | 120.0 | 124.1 | 133.0 | 100.0 | 144.0 | 124.2 | 150.0 | 156.5 | 150.0 | 100.0 | 156.5 | 142.7 |
| 17 | 67.5 | 82.5 | 28.0 | 7.6 | 46.4 | 13.77 | 41.09 | 40.30 | 51.36 | 36.63 | 29.04 | 59.75 | 46.63 | 53.08 | 47.12 | 50.8 | 60.4 | 48.6 | 21.4 | 34.0 | 39.2 | 163.2 | 150.0 | 107.4 | 20.0 | 72.7 | 102.7 |
| 22 | 155.8 | 137.3 | 91.2 | 74.2 | 114.6 | 7.87 | 22.43 | 14.62 | 31.21 | 19.03 | 43.11 | 53.49 | 35.25 | 47.99 | 44.95 | 116.1 | 109.0 | 109.0 | 61.4 | 126.3 | 104.4 | 159.0 | 151.2 | 144.0 | 100.0 | 163.6 | 143.6 |
| 23 | 81.7 | 119.2 | 69.3 | 47.4 | 79.4 | 11.77 | 33.04 | 29.91 | 39.66 | 28.59 | 30.25 | 59.00 | 45.70 | 50.38 | 46.33 | 85.7 | 85.7 | 82.7 | 30.0 | 71.1 | 71.0 | 147.0 | 153.2 | 150.0 | 100.0 | 167.4 | 143.5 |

Here, again, the marked effect of manure, particularly in the case of corn and wheat, is evident. These two plots rank at the bottom of the series when untreated, but by the addition of manure they are moved from ninth and tenth places to first and second, manure having a much greater influence on these than on any other plots studied. The effect of manure is quite marked on the timothy and rotation plots, though not nearly so great as in the case of corn and wheat. Considering both seasons, we find the percentage of increase caused by the application of manure to be as follows:

| | | 1914 | 1913 |
|---|---|---|---|
| Corn | per cent. | 217 | 160 |
| Wheat | per cent. | 150 | .... |
| Timothy | per cent. | 47 | 44 |
| Rotation | per cent. | 28 | 32 |

The increases due to the application of chemicals were as follows: Wheat, 103 per cent; rotation, 30 per cent.

These figures are from the average of those samples receiving no calcium carbonate. Considering the same phenomenon when calcium carbonate was added to the test samples, we find entirely different data: Corn, 39 per cent; wheat, 21 per cent; timothy, no increase; rotation, 9 per cent. When chemicals were added the increase of wheat was 9 per cent; that of the rotation plot, 4 per cent.

The application of calcium carbonate to the soil from different plots in 1914 gives some equally interesting data:

| Plot No. | Cropping system. | Percentage of increase due to calcium carbonate. |
|---|---|---|
| 17 | Continuous corn | 162 |
| 18 | Continuous corn, with the addition of manure | 15 |
| 9 | Continuous wheat | 187 |
| 10 | Continuous wheat, with the addition of manure | 40 |
| 2 | Continuous wheat, with the addition of chemicals | 54 |
| 23 | Continuous timothy | 102 |
| 22 | Continuous timothy, with the addition of manure | 37 |
| 13 | Six-year rotation | 89 |
| 1 | Six-year rotation, with the addition of manure | 61 |
| 3 | Six-year rotation, with the addition of chemicals | 50 |

Calculating from the 1914 tests the percentage increase in the nitrate formation of manured soil with the addition of calcium carbonate over the untreated in the absence of calcium carbonate, we approximate the combined influence of both factors. Estimating the theoretical effect of the two factors by adding the increases resulting from manure alone and from calcium carbonate alone, very different results are obtained.

From these data and those given above it is evident that in only one case of the series studied is it possible to replace entirely the calcium carbonate by manure or the manure by calcium carbonate. However, this may be done to a very large extent. In the case of timothy it is not

possible to replace calcium carbonate with manure. The effect of manure, however, can be entirely eliminated by calcium carbonate. As perhaps would be expected, the percentage increases due to calcium carbonate not replaceable by manure are in an inverse ratio to those of manure not replaceable by calcium carbonate. Such increases seem to be, in general, correlated with the type of crop—that is, those crops naturally depleting the soil of organic matter (corn and wheat) show a large percentage increase from manure not replaceable by calcium carbonate, while those naturally keeping up the organic matter (rotated) show a larger percentage due to calcium carbonate not replaceable by manure.

| Cropping system. | Percentage of increase due to manure and calcium carbonate. | | Difference. |
|---|---|---|---|
| | Actual. | Calculated. | |
| Corn | 264 | 379 | 115 |
| Wheat | 247 | 337 | 90 |
| Timothy | 102 | 149 | 47 |
| Rotation | 107 | 117 | 10 |

It seems evident that in a general way the effect of the two agencies are the same, so far as nitrate formation is concerned. This does not support Temple's contention (20) that the beneficial effect of manure is due to organisms actually brought in with the manure.

| Cropping system. | Percentage of increase due to calcium carbonate in presence of manure or not replaceable by it. | Percentage of increase due to manure in presence of calcium carbonate or not replaceable by it. |
|---|---|---|
| Corn | 15 | 39 |
| Wheat | 40 | 21 |
| Timothy | 37 | 0 |
| Rotation | 61 | 9 |

It is also interesting to note that in the rotation studied either with or without the addition of calcium carbonate, the effect on nitrate formation of the chemicals has been practically identical with that of the manure. In the case of wheat, chemicals have had only about one-half of the beneficial effect that manure has had. As to the effects of the different crops, corn and wheat undoubtedly have a harmful effect in the absence of manure, both ranking very low either with or without calcium carbonate. On the other hand, with the addition of manure they are raised from tenth and ninth places to first and second places, respectively. Timothy and the rotation are approximately equal and very much higher than corn

and wheat. The effect of manure, however, was not very marked on either, being somewhat more pronounced in the case of timothy. When calcium carbonate was added to the test samples the only noticeable effect of the crop is the low position held by corn and wheat in absence of manure.

The fact that the addition of calcium carbonate to samples eliminates to a large extent the very large and unmistakable differences, otherwise detectable, raises the question as to which method probably more accurately represents field conditions. We shall only call attention to the fact that Löhnis and Green (11) vigorously maintain that the addition of calcium carbonate is essential, while Temple (21) has shown that with organic sources of nitrogen vigorous nitrification is possible even in acid soils. Table VI gives the results of an experiment to determine the effect of varying the quantity of nitrogen and calcium carbonate added. This test was run in order to determine the specific quantity of calcium carbonate necessary to insure maximum nitrification and also the correct amount of nitrogen to be added.

TABLE VI.—*Effect on nitrate formation of varying the quantity of calcium carbonate and nitrogen added to soil*

| Plot 17. | | | | | Plot 18. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Calcium carbonate. | Nitrogen as cotton-seed meal. | Nitrate. | Nitrogen as ammonia. | Nitrate. | Calcium carbonate. | Nitrogen as cotton-seed meal. | Nitrate. | Nitrogen as ammonia. | Nitrate. |
| *Gm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Gm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| [a] 0 | 60 | 60 | 60 | 13.7 | [a] 0 | 60 | 104.3 | 60 | 43.2 |
| [a] .05 | 60 | 75.8 | 60 | 23.4 | [a] .05 | 60 | 133.3 | 60 | 66.4 |
| [a] .10 | 60 | 82.7 | 60 | 34.2 | [a] .10 | 60 | 124.7 | 60 | 85.2 |
| [a] .25 | 60 | 94.2 | 60 | 42.0 | [a] .25 | 60 | 135.8 | 60 | 156.5 |
| [a] .50 | 60 | 146.9 | 60 | 55.3 | [a] .50 | 60 | 144.0 | 60 | 218.1 |
| [a] 1.00 | 60 | 141.9 | 60 | 74.0 | [a] 1.00 | 60 | 138.4 | 60 | 232.2 |
| [a] 2.50 | 60 | 144.0 | 60 | 70.8 | [a] 2.50 | 60 | 162.8 | 60 | 244.0 |
| [a] 5.00 | 60 | 144.0 | 60 | 80.0 | [a] 5.00 | 60 | 135.8 | 60 | 244.0 |
| [b] 1 | 0 | 22.3 | 0 | 28.2 | [b] 1 | 0 | 32.5 | 0 | 31.5 |
| [b] 1 | 15 | 56.8 | 15 | 60.0 | [b] 1 | 15 | 58.3 | 15 | 80.0 |
| [b] 1 | 30 | 85.7 | 30 | 75.0 | [b] 1 | 30 | 92.3 | 30 | 108.2 |
| [b] 1 | 60 | 144.0 | 60 | 90.0 | [b] 1 | 60 | 142.8 | 60 | 266.6 |
| [b] 1 | 120 | 193.5 | 120 | 37.2 | [b] 1 | 120 | 244.0 | 120 | 369.0 |
| [b] 1 | 240 | 8.8 | 240 | 7.8 | [b] 1 | 240 | 444.4 | 240 | 232.2 |
| [c] 0 | 0 | 14.0 | 0 | 14.0 | [c] 0 | 0 | 20.0 | 0 | 20.0 |

[a] Nitrogen constant, calcium carbonate varying.
[b] Calcium carbonate constant, nitrogen varying.
[c] Nitrate originally in soil.

The amount of nitrate formed in seven days is so insignificant that we have left the 7-day data out of consideration. It is interesting, however, to note that those plots which rank high for 28 days also rank high for 7 days.

A study of the relative position of the different plots at the various analyses shows clearly that the method used is reliable for detecting

differences, whether the nitrate-forming power is low or high. Table VII gives the relative rank of the different plots at each analysis. Figure 1 illustrates very clearly the low and the high nitrate-forming power at different periods.

TABLE VII.—*Average nitrate formation without addition of calcium carbonate (Table V) of seven fertility plots studied in 1913 and 1914, together with their relative rank at each sampling*

| Plot No. | 1913 | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Average nitrate formation. | Rank. | | | | Average nitrate formation. | Rank. | | | | |
| | | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. |
| | *Mgm.* | | | | | *Mgm.* | | | | | |
| 1 | 94.7 | 5 | 4 | 4 | 4 | 91.5 | 4 | 4 | 4 | 3 | 4 |
| 10 | 104.5 | 4 | 2 | 1 | 2 | 107.2 | 2 | 2 | 3 | 2 | 3 |
| 13 | 71.9 | 3 | 7 | 5 | 6 | 71.5 | 5 | 5 | 6 | 5 | 5 |
| 17 | 46.4 | 7 | 6 | 7 | 7 | 39.2 | 7 | 7 | 7 | 7 | 7 |
| 18 | 120.2 | 2 | 1 | 2 | 1 | 124.2 | 1 | 1 | 1 | 1 | 1 |
| 22 | 114.6 | 1 | 3 | 3 | 3 | 104.4 | 3 | 3 | 2 | 4 | 2 |
| 23 | 79.4 | 6 | 5 | 6 | 5 | 71.0 | 6 | 6 | 5 | 6 | 6 |

Since the addition of calcium carbonate has to such a large extent eliminated the differences in nitrate formation when testing in the soil itself, it is interesting to ascertain whether this can be traced to an elimination of acid conditions. Dr. P. F. Trowbridge, of the Department of Agricultural Chemistry, Missouri Experiment Station, has furnished us with the following data regarding the lime requirements of some of the plots. The figures are in pounds per acre-foot; basis, 3,000,000 pounds per acre: Plot 2, 7,900; plot 13, 7,200; plot 17, 7,900; plot 18, 2,400; plot 22, 2,400; plot 23, 7,200 pounds.

It will be noted that, so far as the crop is concerned, the differences in lime requirements are very slight (plots 13, 17, and 23 or 18 and 22). Manure kept the acidity low (plots 18 and 22). Commercial fertilizers have had no appreciable effect upon acidity (plot 2). Nevertheless, continuous corn soil with a lime requirement not materially different from that of timothy, the rotation, or continuous wheat receiving commercial fertilizers produced only about one-half the amount of nitrates as these soils. It will be shown later that transferring the organisms to a common pabulum containing an abundance of calcium carbonate does not eliminate the large differences. In this connection, as a suggested explanation of one of the effects of lime when added to the soil, we call attention to the work of Schreiner and Reed (16), who have demonstrated the stimulative effect of calcium carbonate upon oxidases. It is not impossible that soil conditions are sometimes such as to permit the accumulation of nitrate-forming oxidases, while other conditions

will not permit similar accumulations. The work of the Bureau of Soils has also demonstrated that calcium carbonate is rather efficient in eliminating the toxic effect of certain decomposition products found in soils, and the beneficial effect might, in part, be due to an action of this nature.

The following data were obtained during 1913 concerning the relative effect of 6 years' application of manure compared with that for 25 years: Plot 29, continuous wheat, received manure for 6 years and produced in one week's incubation an average of 5.35 mgm. of nitrate; in four weeks an average of 116.6 mgm. In the inoculation experiments it produced 15.3 mgm. Plot 30 received manure for 25 years, otherwise similar to No. 29, and produced in one week 6.5 mgm. of nitrate; in four weeks 127 mgm., and in inoculating experiments, 27.5 mgm. This indicates that the shorter period of application has produced almost the same effect as the longer.

## NITRIFYING INOCULATION EXPERIMENTS

The results of the nitrifying inoculation experiments are reported in Table VIII. It should be borne in mind that the 1913 results were obtained under conditions not favorable for nitrate formation, no calcium carbonate having been added. Therefore, too much weight should not be attached to these results. The 1914 results, however, were secured under favorable conditions. It is worthy of note, though, that there is a very close agreement between the relative rank of the plots in the inoculating experiments for both seasons and the nitrate-forming experiments. This is shown in Table IX, together with the relative position of bacterial numbers.

The agreement is not absolute, but it is close enough to indicate the probability of the same factors controlling the nitrate formation in the two instances. This being true, the factors must be biological in nature rather than chemical or physical; otherwise they probably would not be transferred in 20 c. c. of a 2 to 1 soil suspension in sufficient quantities to exercise much influence. There is also exhibited here a close correlation between bacterial numbers and nitrate formation. Since, however, no such correlation can be traced in ammonia formation, there is little reason for believing the two factors connected other than that probably the factors controlling both are the same.

TABLE VIII. — *Ammonia and nitrate formation (in milligrams) in soil X when inoculated with soil from different fertility plots during 28 days' incubation.*

| Inoculum plot No. | 1913 | | | | | | | | | | | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrate ($NO_3$) recovered. | | | | | Nitrogen as ammonia recovered. | | | | | Nitrogen as nitrate and ammonia recovered. | | | | | Nitrate ($NO_3$) recovered. | | | | | |
| | Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 34.2 | 31.6 | 0 | 0 | 16.4 | 37.02 | 35.66 | 47.68 | 29.96 | 37.58 | 44.76 | 42.81 | 47.68 | 29.96 | 41.30 | 125.2 | 54.0 | 41.6 | 54.0 | 70.5 | 69.1 |
| 13 | 5.7 | 32.6 | 0 | 0 | 9.6 | 48.81 | 33.25 | 49.19 | 33.01 | 41.06 | 50.10 | 40.39 | 49.19 | 33.01 | 43.17 | 65.4 | 10.3 | 14.3 | 20.3 | 25.7 | 27.2 |
| 3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 150.0 | 55.3 | 65.2 | 56.2 | 81.8 | 81.7 |
| 10 | 16.2 | 14.7 | 7.0 | 2.0 | 10.0 | 49.63 | 30.57 | 47.03 | 28.72 | 38.99 | 53.29 | 33.89 | 48.61 | 29.17 | 41.25 | 180.0 | 53.3 | 63.1 | 78.2 | 90.1 | 92.9 |
| 9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 37.1 | 5.0 | 7.5 | 7.4 | 5.2 | 12.4 |
| 2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 156.6 | 83.0 | 55.5 | 34.8 | 44.4 | 74.9 |
| 18 | 76.7 | 55.1 | 4.7 | 3.3 | 35.0 | 28.40 | 26.74 | 37.93 | 29.96 | 30.76 | 45.76 | 39.20 | 38.99 | 30.69 | 38.67 | 180.0 | 82.7 | 58.2 | 64.3 | 161.1 | 109.3 |
| 17 | 0 | 14.4 | 0 | 0 | 3.6 | 48.36 | 36.02 | 47.68 | 26.28 | 39.58 | 48.36 | 39.27 | 47.68 | 26.28 | 40.38 | 80.0 | 9.8 | 5.3 | Trace. | 6.3 | 20.3 |
| 22 | 23.3 | 38.2 | 0 | 3.2 | 16.2 | 44.38 | 28.02 | 44.64 | 24.45 | 35.37 | 49.65 | 36.66 | 44.64 | 25.17 | 39.03 | 141.7 | 35.0 | 46.1 | 38.5 | 69.6 | 66.2 |
| 23 | 4.0 | 7.0 | 0 | 0 | 2.7 | 52.29 | 32.69 | 46.60 | 19.92 | 37.82 | 53.19 | 34.27 | 46.60 | 19.92 | 38.49 | 93.0 | 10.6 | 33.3 | 20.2 | 8.6 | 33.1 |

TABLE IX.—*Comparison of the ranks in 1913 and 1914 of the bacterial numbers and nitrate-forming and inoculation experiments*

| Plot No. | 1913 | | | 1914 | | |
|---|---|---|---|---|---|---|
| | Bacterial numbers. | Nitrate-forming experiments. | Inoculation experiments. | Bacterial numbers. | Nitrate-forming experiments. | Inoculation experiments. |
| 1.... | 4 | 4 | 2 | 4 | 4 | 3 |
| 13... | 5 | 6 | 5 | 6 | 5 | 6 |
| 10... | 1 | 3 | 4 | 2 | 2 | 2 |
| 18... | 1 | 1 | 1 | 1 | 1 | 1 |
| 17... | 7 | 7 | 6 | 7 | 7 | 7 |
| 22... | 3 | 2 | 3 | 3 | 3 | 4 |
| 23... | 6 | 5 | 7 | 6 | 6 | 5 |

## CROSS-INOCULATION EXPERIMENTS

Unfortunately, time permitted the conducting of only one experiment along the line of cross-inoculation. An effort was made to make this as representative as possible, both by using as the pabulum a mixture of all samples collected from the respective plots during both seasons and by inoculating in duplicate sterile samples, containing 60 mgm. of nitrogen as cottonseed meal from every other plot. In addition, calcium carbonate was added to one of the duplicates. The results are reported in Table X.

Reading horizontally, one obtains figures representing the capacity—using this term in the sense that Stevens and Withers (17) used it—of soil from the different plots to support nitrification both with and without the addition of calcium carbonate. It is noted that all the soils will support vigorous nitrification, provided vigorous nitrifiers are added. If the averages in the last column are examined, it will be noted that when calcium carbonate is added differences can be noted, but they are not so marked as some other results, the highest figure being 99.9 and the lowest 72.7. In the absence of calcium carbonate the figures are not nearly so high, and, though the differences are somewhat more marked, the relative positions are not materially different from those where calcium carbonate was added. Examining any vertical column, we obtain figues representing the ability of the various soils to support the nitrifying flora from any one particular soil; or inversely, the ability of any particular soil to inoculate the others. This also varies materially, indicating that the soil does exercise a marked influence upon nitrification, some floras thriving better in one soil and other floras better in others. Considering the average of the vertical columns, we obtain what may be termed the "relative inoculating ability." Here we obtain our greatest variation, indicating, as previously suggested, that to the flora itself must be ascribed the major differences in nitrification. Here again, the continuously cropped plots, wheat and corn, rank lowest when no manure is added; but manure exercises a greater influence on them than any other in the series. In the case of timothy, manure has not increased its inoculating ability; in fact, the reverse is true, while with the rotated plot the effect has not been nearly so great as with corn and wheat.

TABLE X.—*Results of cross-inoculation soil experiments expressed in milligrams of nitrate per 100 gm. of soil*

| Plot No. | Nitrate ($NO_3$), with addition of calcium carbonate. | | | | | | | | | | | Nitrate ($NO_3$), without addition of calcium carbonate. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inoculum..... | 1 | 2 | 3 | 9 | 10 | 13 | 17 | 18 | 22 | 23 | Average. | 1 | 2 | 3 | 9 | 10 | 13 | 17 | 18 | 22 | 23 | Average. |
| 1..... | 72.0 | 108.0 | 108.0 | 0 | 180.0 | 86.4 | 24.0 | 112.5 | 36.6 | 83.7 | 81.1 | 36.0 | 33.5 | 8.3 | 0 | 45.9 | 12.2 | 0 | 35.4 | 0 | 12.6 | 18.4 |
| 13..... | 116.1 | 100.0 | ..... | 0 | 109.0 | 75.0 | 26.1 | 116.1 | 85.7 | 92.3 | 80.0 | 21.0 | 31.3 | 14.6 | 0 | 56.2 | 33.2 | 6.2 | 25.7 | 6.6 | 22.5 | 21.7 |
| 3..... | 124.1 | 63.5 | 56.8 | 0 | 171.4 | 83.8 | 9.0 | 112.5 | 25.7 | 80.0 | 72.7 | 31.7 | 32.7 | 7.6 | 0 | 69.6 | 15.0 | 0 | 24.8 | 0 | 9.2 | 19.1 |
| 10..... | 150.0 | ..... | 128.5 | 4.0 | 180.0 | 94.7 | 17.4 | 112.5 | 109.0 | 69.2 | 96.2 | 60.0 | ..... | 12.8 | 0 | 40.0 | 29.1 | 4.1 | 33.7 | 7.8 | 20.0 | 20.7 |
| 9..... | 120.0 | 90.0 | 56.8 | 0 | 163.6 | 86.4 | 30.8 | 128.5 | 38.5 | 62.0 | 77.7 | 27.1 | 25.7 | 5.1 | 0 | 30.8 | 18.0 | 0 | 27.3 | 0 | 7.8 | 14.2 |
| 2..... | 128.5 | 138.4 | 94.7 | 0 | 133.3 | 43.2 | 6.0 | 85.7 | 38.5 | 85.7 | 75.4 | 34.8 | 46.9 | 8.1 | 0 | 31.3 | 16.3 | 0 | 28.8 | 0 | 11.6 | 17.8 |
| 17..... | 94.7 | 97.3 | 105.8 | 0 | 133.4 | 87.8 | 30.8 | 81.8 | 46.9 | 54.5 | 73.3 | 12.0 | 15.6 | 3.9 | 0 | 27.7 | 23.3 | 5.0 | 23.2 | 0 | 17.1 | 12.8 |
| 18..... | 144.0 | 120.0 | 100.0 | 0 | 144.0 | 87.8 | 42.3 | 128.5 | 60.0 | 94.7 | 92.1 | ..... | 65.4 | 15.6 | 0 | 75.0 | 42.3 | 13.3 | 63.1 | 4.8 | 27.6 | 34.1 |
| 22..... | 128.5 | 133.3 | 109.0 | 0 | 150.0 | 150.0 | 46.9 | 144.0 | 21.2 | 94.7 | 97.8 | 25.7 | 56.8 | 17.1 | 0 | 76.5 | 45.9 | 9.7 | 76.5 | 5.3 | 30.4 | 34.4 |
| 23..... | 156.5 | 86.4 | 144.0 | 0 | 180.0 | 138.4 | 45.0 | 150.0 | 52.6 | 45.9 | 100.8 | 43.2 | 25.4 | 12.4 | 0 | 76.5 | 50.2 | 7.2 | 54.0 | 0 | 28.2 | 29.7 |
| Average. | 137.1 | 104.1 | 100.4 | 0.4 | 154.4 | 93.3 | 27.8 | 117.2 | 51.5 | 76.3 | ..... | 32.4 | 37.0 | 10.5 | 0 | 52.9 | 28.5 | 4.5 | 39.3 | 2.4 | 18.7 | ..... |

The nitrifying capacity averages in the last column of Table X place timothy at the top of the list, manure in this case having no effect. Next in order are wheat and corn, where manure is added. Manure had no increasing effect on the rotated plot. The continuously cropped plots without manure, the rotated plots, and the plots receiving chemicals vary little. Manure here exercises little or no influence on those crops not depleting the soil of organic matter.

It is evident that the nitrifying floras of plots 9, 17, and 22, the first two especially, were extremely weak. Though these plots possessed at this time good nitrate-forming floras, they were not able to overcome the adverse effects experienced in transferring them to soils slightly less favorable but in which a more vigorous or differently constituted flora thrived.

### NITRATE NITROGEN UNDER FIELD CONDITIONS

In Table XI is given the quantity of nitrogen as nitrate per 100 gm. of soil when the soil was collected from the field, but nothing particularly marked is to be noted from the results given. As would be expected, this quantity varies considerably; but since the demands made upon the various plots by the growing crop differ widely, little information is furnished regarding the rate of formation. After the wheat is harvested the nitrate content of wheat plots increases rapidly. Wheat was cut on June 20, 1913, and on June 28, 1914. Some accumulation is evident even before harvest. When wheat is growing rapidly no nitrates are present. The accumulation is much more marked in the presence of manure and chemicals than in their absence. There is an abundance of nitrate nitrogen under corn even when it is making its most rapid growth. During 1913 the water content fell so low in July and August that no nitrates could be formed; with rain coming in October, however, the nitrate content rose rapidly. In all cases where a comparison of the same crop in the presence and absence of manure is possible the nitrate content of the manure plot is materially higher than that of the unmanured.

TABLE XI.—*Quantity (in milligrams per 100 grams of soil) of nitrate in soil when sampled from fertility plots at Columbia, Mo., in 1913 and 1914* [a]

| Plot No. | Quantity of nitrate ($NO_3$). | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1913 | | | | | | 1914 | | | | | |
| | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 3.91 | 2.06 | 10.66 | 7.71 | 2.72 | 5.41 | 1.94 | 3.20 | 4.00 | 1.50 | 1.60 | 2.45 |
| 13 | 1.43 | .75 | 1.07 | 1.90 | 1.25 | 1.28 | 2.10 | 1.13 | 2.10 | 0.75 | Trace | 1.22 |
| 3 | | | | | | | 0 | 1.27 | 2.00 | 1.20 | 1.50 | 1.19 |
| 10 | 6.40 | 8.78 | 18.00 | 11.08 | Trace. | 8.85 | 1.97 | 3.60 | 8.50 | 6.40 | 5.00 | 5.09 |
| 9 | | | | | | | 0 | 1.71 | 5.50 | 4.50 | 3.00 | 2.94 |
| 2 | | | | | | | 0 | 2.70 | 4.80 | 4.50 | 7.50 | 3.90 |
| 18 | 4.48 | .75 | 4.46 | 5.67 | 3.57 | 3.79 | 2.40 | 6.00 | 12.20 | 7.20 | 2.50 | 6.06 |
| 17 | 2.48 | .66 | .53 | 2.10 | 3.06 | 1.76 | 3.10 | 6.12 | 6.43 | 1.70 | 1.90 | 3.85 |
| 22 | 1.94 | 1.91 | 6.50 | 1.90 | Trace. | 2.45 | 2.10 | 1.80 | 3.60 | 5.40 | 3.10 | 3.20 |
| 23 | 1.38 | 1.28 | 2.73 | Trace. | Trace. | 1.08 | 0 | 0 | 1.80 | 2.90 | 1.20 | 1.18 |

[a] Soil was dried at 110° C. for two hours.

## SUMMARY

(1) The agricultural methods practiced upon the plots under study have brought about marked differences in the number of organisms contained in the soil, at least those capable of developing under our experimental conditions. The soil under continuous corn and wheat contains, in the absence of any additions of fertilizers or manure, relatively low numbers of bacteria. In the presence of manure, continuous corn and wheat soil contain relatively high numbers, manure having a much more marked effect upon numbers here than under the other crops studied.

(2) The agricultural practices under study have, so far as we can detect without experimental methods, produced no appreciable effect upon the ability of the soil and its organic life to liberate ammonia from cottonseed meal.

(3) The ability of the soil complex to oxidize ammonia nitrogen to nitrate nitrogen has been materially altered by the methods under study. This we believe to be due in part to physical and chemical changes in the soil and in part to biological changes. Continuous corn and wheat with no additions of manure or chemicals have brought about a relative low oxidizing power in the soil complex. The addition of manure materially raises the oxidizing power, especially under continuous corn and wheat. The addition of commercial fertilizer brings about a condition similar to that of manure, though perhaps less marked.

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# STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL.—XV. DWARF EGGS[1]

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## INTRODUCTION

Eggs much smaller than normal eggs are occasionally produced by domestic fowls of all breeds. These eggs usually contain little or no yolk; but occasionally a small yolk, usually without germ disk but inclosed in a complete vitelline membrane, is present. The albumen is small in amount, and often but not always it is of a thicker consistency than the albumen of a normal egg. The egg membranes are normal. The shell varies in thickness over the same range as the shells of normal eggs. Sometimes, as in eggs otherwise normal, shell is entirely lacking—that is, the egg is simply covered with a membrane.

These small eggs are called by various names as "cock eggs," "witch eggs," "luck eggs," "wind eggs," "dwarf eggs," etc. Most of these names are associated with interesting superstitions. The term used by the people in any particular part of the world depends in part on the folklore of the region. Since no single term is generally accepted, we have decided to use a name which, although it has no legendary history, is somewhat descriptive. We have therefore called these small eggs "dwarf eggs."

Among the various types of abnormal eggs produced by the domestic fowl the dwarf egg is more common than any other type except the double-yolked egg. This type of egg has played an important rôle in the folklore of all nations. Sebillot (22),[2] Tiedeman (25), König-Warthausen (7), and Bonnet (2) give some of the popular superstitions connected with these eggs. A widespread superstition which comes down nearly to our own time is that a cock, or especially a very old cock, produces these eggs. These "cock" eggs were sometimes supposed to be made up of semen and "humors." A superstition which was quite widely accepted at an early period was that such an egg might hatch into a fabled

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 98.
[2] Reference is made by numbers to "Literature cited," p. 1041.

serpent, the basilisk, whose breath or look was fatal. The basilisk was sometimes said to have head and legs like a cock. Less definite superstitions which simply regard the "cock" egg as an evil omen have also been common. In some places European peasants cast these eggs behind them over a wall or building to avoid bad luck. In other places they used them as evil charms to avenge themselves on their enemies. A very mild sort of vengeance practiced in some localities was to place one of these eggs among the eggs belonging to a neighbor. This prevented his eggs from hatching. A more violent charm was made by breaking the dwarf egg and filling part of the shell with dew collected at dawn from a white thorn tree and then exposing this to the sun. A terrible calamity was supposed to happen to the designated person as the sun drank the last drop of dew.

On the other hand, these eggs have been considered as a sign of good luck. Pearl (11) reported such a superstition which only a few years ago was accepted by a few credulous country people in some parts of the United States. According to this version of the myth a "luck egg" does not break when thrown over a building, and any wish made by the thrower while the egg is in the air is sure to come true.

The dwarf, witch, or cock egg has emerged from the age of superstition with the cause for its production inadequately explained. It is the purpose of the present paper to discuss (1) the different types of dwarf eggs in respect to shape and also in respect to contents; (2) the variability in respect to size and shape; (3) the interrelations of the variations in dimensions, shape, and size; (4) the frequency of the occurrence of dwarf eggs compared to normal eggs and of dwarf-egg producers compared to birds which do not lay dwarf eggs; (5) the seasonal distribution of dwarf eggs; (6) dwarf-egg production by birds with normal and with abnormal oviducts; (7) the relation of dwarf-egg production by normal birds to the age of the bird and to the position of the egg in the clutch and litter; (8) physiological conditions which lead to dwarf-egg production; (9) the relation of the production of dwarf eggs to other abnormal phenomena of reproduction which either occur in nature or have been experimentally produced; and (10) the contribution which the study of the physiology of dwarf-egg production makes to our knowledge of the normal physiology of egg production.

Since February, 1908, the abnormal eggs laid at the poultry plant of the Maine Agricultural Experiment Station have been brought to the laboratory for examination. In the eight years to February, 1916, 298 dwarf eggs are known to have been produced at this plant. The weight of 275 of these was taken, and in 261 of these cases the length and breadth were also measured and the length-breadth index calculated. Of the 298 eggs recorded 274 were opened, and their contents were examined. Several of the dwarf eggs were floor eggs and a few were laid by birds on which no egg record was kept. In 251 cases, however, the egg record

of the bird laying the dwarf egg is available. Furthermore, autopsies were made on several of these birds, and the condition of their sex organs was observed.

## I.—CLASSIFICATION OF DIFFERENT TYPES OF DWARF EGGS, FIRST, IN RESPECT TO SHAPE, AND SECOND, IN RESPECT TO PRESENCE OR ABSENCE OF YOLK

The dwarf eggs of the fowl vary greatly in size and shape. Plate CXII shows 14 of these eggs with a normal egg laid by a 9-months-old pullet for comparison. From this illustration it may be seen that there are two distinct types of dwarf eggs in respect to their shape: The prolate-spheroidal type, similar in shape to a normal egg; and the cylindrical type, which is much longer in proportion to the breadth. The cylindrical eggs are shown in the first row of Plate CXII. These cylindrical eggs occur much less frequently than do the dwarf eggs of the prolate-spheroidal type. Of the 261 dwarf eggs on which complete data are available only 12, or 4.6 per cent, were of this form.

Not only do the dwarf eggs differ in respect to size and shape but there is a difference in internal structure. These dwarf eggs are sometimes defined as yolkless eggs, or small eggs containing a small quantity of yolk usually not in a yolk membrane. During this investigation the contents of 274 dwarf eggs were examined. It was found that some of these eggs contained no yolk but appeared to be formed around a nucleus which consisted of a few strings of coagulated albumen, apparently untwisted chalazal threads and also sometimes small lumps of hardened albumen or small blood clots. On the other hand, some contain small yolks in yolk membranes. These yolks do not usually have visible germ disks. The weight of these yolks varies from 1 gm. to nearly 8 gm. More than half of all the eggs opened, however, contained some yolk which was not inclosed in a yolk membrane. Dwarf eggs may then be classified according to the nonoccurrence of yolk and the condition of the yolk when present as, first, yolkless, second, with some yolk not in a membrane, and third, with one small yolk. In Table I the dwarf eggs are classified both according to form and yolk content.

TABLE I.—*Classification of dwarf eggs both as to shape and as to yolk content*

| Shape. | Number yolkless. | Percentage yolkless. | Number with some yolk not in a membrane. | Percentage with some yolk not in a membrane. | Number with one small yolk. | Percentage with one small yolk. | Total. |
|---|---|---|---|---|---|---|---|
| Shape not known [a] | 5 | 38.46 | 8 | 61.54 | 0 | 0 | 13 |
| Prolate-spheroidal shape | 83 | 33.33 | 139 | 55.82 | 27 | 10.84 | 249 |
| Cylindrical shape | 8 | 66.67 | 4 | 33.33 | 0 | 0 | 12 |
| Total | 96 | 35.03 | 151 | 55.11 | 27 | 9.85 | 274 |

[a] Dimensions not recorded.

From the last line of Table I it is seen that 96, or 35.03 per cent, of the eggs opened were yolkless. The other 178, or 64.96 per cent, contained yolk. Of these, 151, or 55.11 per cent, of all the dwarf eggs opened contained yolk not inclosed in a yolk membrane. A small yolk was present in 27, or 9.85 per cent, of the dwarf eggs. From these figures it is seen that nearly two-thirds of the dwarf eggs contain yolk.

## II.—THE ALBUMEN AND SHELL OF DWARF EGGS

We have seen that dwarf eggs differ in respect to the nucleus around which the albumen is formed. Bonnet (3) states that the nature of the albumen is also generally altered. The dwarf eggs observed differed greatly in respect to the density of the albumen. In many it was very condensed, being a thick clear mass which nearly maintained its shape when removed from the shell and egg membranes. It appeared very much like the albumen in a normal egg while it is in the albumen-secreting region, or the isthmus of the oviduct (15). In many other cases it appeared exactly like the albumen of a normal laid egg—that is, there was a somewhat firm inner mass surrounded by a thin fluid albumen. All gradations between these also occurred. In a very few cases the albumen was more fluid than in the average normal egg. There was, however, an undoubted general tendency for the albumen to be more than normally firm. The density of the albumen was not determined accurately as a routine procedure in the dwarf eggs. Its apparent density as compared to normal eggs was frequently, but unfortunately not uniformly, recorded. In connection with another investigation in progress at this laboratory the specific gravity and refractive index of the albumen of many normal and a few dwarf eggs was determined. These probably were not a random sample of dwarf eggs. The minimum specific gravity of the sample of 7 dwarf eggs was 1.02824, while the mean specific gravity for the sample of 180 normal eggs was 1.0288. The dwarf eggs ranged widely, with the upper end of the range decidedly above that for normal eggs. The maximum for dwarf eggs was 1.2107, against a maximum of 1.0415 for the normal eggs. The mean for the dwarf eggs was 1.0627, which is higher than the maximum for normal eggs. The range, however, overlaps, 4 of the 7 dwarf eggs falling within the upper end of the range for normal eggs. In a sample of 10 dwarf eggs the refractive index lay within the range for the sample of 180 normal eggs. The mean was slightly higher for the dwarf than for the normal eggs; but this difference certainly was not significant.

The egg membranes of dwarf eggs, so far as superficial appearances indicate, are comparable to those of normal eggs. The shell is sometimes entirely or almost entirely absent, as in the case of membrane-covered or soft-shelled eggs, which are normal in all other particulars. The thickness of shell varies from very thin to very thick, as in normal eggs.

In the present investigation no further distinction is made between dwarf eggs in respect to variation in albumen or shell.

## III.—SIZE AND SHAPE RELATIONS OF THE SEVERAL CLASSES OF DWARF EGGS COMPARED TO EACH OTHER AND TO NORMAL EGGS AND THE RELATIVE VARIABILITY OF NORMAL AND OF THE DIFFERENT CLASSES OF DWARF EGGS

There is a considerable amount of variation within each class of dwarf eggs in respect to every measurable character. Table II gives for each class the frequency distribution of variation for each dimension, the shape index, and the weight.

TABLE II.—*Frequency distributions of the variation in size and shape of the several classes of dwarf eggs*

LENGTH

| Class. | Frequency of prolate-spheroidal shape. | | | Frequency of cylindrical shape. | |
|---|---|---|---|---|---|
| | Yolkless. | Some free yolks. | A small yolk. | Yolkless. | Some free yolk. |
| *Mm.* | | | | | |
| 20. 0–22. 9 | 1 | 1 | ..... | ..... | 1 |
| 23. 0–25. 9 | 2 | 1 | ..... | ..... | 0 |
| 26. 0–28. 9 | 5 | 11 | ..... | 1 | 0 |
| 29. 0–31. 9 | 14 | 32 | ..... | 0 | 2 |
| 32. 0–34. 9 | 16 | 25 | 1 | 0 | 1 |
| 35. 0–37. 9 | 23 | 16 | 6 | 3 | ..... |
| 38. 0–40. 9 | 11 | 23 | 4 | 1 | ..... |
| 41. 0–43. 9 | 6 | 16 | 7 | 0 | ..... |
| 44. 0–46. 9 | 5 | 7 | 7 | 1 | ..... |
| 47. 0–49. 9 | ..... | 5 | 1 | 0 | ..... |
| 50. 0–52. 9 | ..... | 1 | ..... | 1 | ..... |
| 53. 0–55. 9 | ..... | ..... | ..... | 0 | ..... |
| 56. 0–58. 9 | ..... | ..... | ..... | 1 | ..... |
| Total | 83 | [a] 138 | [a] 26 | 8 | 4 |

BREADTH

| | | | | | |
|---|---|---|---|---|---|
| *Mm.* | | | | | |
| 6. 0– 7. 9 | ..... | ..... | ..... | ..... | 1 |
| 8. 0– 9. 9 | ..... | ..... | ..... | ..... | 0 |
| 10. 0–11. 9 | ..... | ..... | ..... | ..... | 0 |
| 12. 0–13. 9 | ..... | ..... | ..... | ..... | 0 |
| 14. 0–15. 9 | ..... | ..... | ..... | ..... | 1 |
| 16. 0–17. 9 | ..... | ..... | ..... | ..... | 1 |
| 18. 0–19. 9 | 1 | 1 | ..... | 1 | 0 |
| 20. 0–21. 9 | 3 | 3 | ..... | 1 | 0 |
| 22. 0–23. 9 | 7 | 5 | ..... | 3 | 1 |
| 24. 0–25. 9 | 7 | 18 | ..... | 0 | ..... |
| 26. 0–27. 9 | 15 | 35 | 1 | 3 | ..... |
| 28. 0–29. 9 | 23 | 25 | 6 | ..... | ..... |
| 30. 0–31. 9 | 20 | 20 | 5 | ..... | ..... |
| 32. 0–33. 9 | 6 | 18 | 5 | ..... | ..... |
| 34. 0–35. 9 | 1 | 9 | 7 | ..... | ..... |
| 36. 0–37. 9 | ..... | 4 | 1 | ..... | ..... |
| 38. 0–39. 9 | ..... | ..... | 1 | ..... | ..... |
| Total | 83 | [a] 138 | [a] 26 | 8 | 4 |

[a] Two dwarf eggs of prolate-spheroidal shape, one with some free yolk and one with a small yolk, were laid after the frequency constants and correlation coefficients had been calculated. These eggs are included in Table I, but not in Table II, III, IV, or V.

TABLE II.—*Frequency distribution of the variation in size and shape of the several classes of dwarf eggs*—Continued.

EGG WEIGHT

| Class. | Frequency of prolate-spheroidal shape. | | | Frequency of cylindrical shape. | |
|---|---|---|---|---|---|
| | Yolkless. | Some free yolks. | A small yolk. | Yolkless. | Some free yolk. |
| *Gm.* | | | | | |
| 3. 0– 5. 9 | 2 | 1 | ..... | 1 | 1 |
| 6. 0– 8. 9 | 6 | 7 | ..... | 0 | 0 |
| 9. 0–11. 9 | 10 | 17 | ..... | 2 | 1 |
| 12. 0–14. 9 | 9 | 34 | 1 | 1 | 2 |
| 15. 0–17. 9 | 21 | 18 | 5 | 0 | ..... |
| 18. 0–20. 9 | 15 | 12 | 2 | 3 | ..... |
| 21. 0–23. 9 | 9 | 15 | 3 | 0 | ..... |
| 24. 0–26. 9 | 5 | 16 | 3 | 1 | ..... |
| 27. 0–29. 9 | 6 | 9 | 6 | ..... | ..... |
| 30. 0–32. 9 | ..... | 3 | 4 | ..... | ..... |
| 33. 0–35. 9 | ..... | 5 | 2 | ..... | ..... |
| 36. 0–38. 9 | ..... | 1 | ..... | ..... | ..... |
| Total | 83 | [a] 138 | [a] 26 | 8 | 4 |

INDEX

| | | | | | |
|---|---|---|---|---|---|
| *Per cent.* | | | | | |
| 34. 0–36. 9 | ..... | ..... | ..... | ..... | 1 |
| 37. 0–39. 9 | ..... | ..... | ..... | ..... | 0 |
| 40. 0–42. 9 | ..... | ..... | ..... | ..... | 0 |
| 43. 0–45. 9 | ..... | ..... | ..... | ..... | 0 |
| 46. 0–48. 9 | ..... | ..... | ..... | 1 | 1 |
| 49. 0–51. 9 | ..... | ..... | ..... | 0 | 1 |
| 52. 0–54. 9 | ..... | ..... | ..... | 1 | 0 |
| 55. 0–57. 9 | ..... | ..... | ..... | 3 | 0 |
| 58. 0–60. 9 | ..... | ..... | ..... | 1 | 1 |
| 61. 0–63. 9 | ..... | ..... | ..... | 1 | ..... |
| 64. 0–66. 9 | 1 | 3 | 1 | 1 | ..... |
| 67. 0–69. 9 | 2 | 5 | 1 | ..... | ..... |
| 70. 0–72. 9 | 3 | 6 | 0 | ..... | ..... |
| 73. 0–75. 9 | 16 | 14 | 2 | ..... | ..... |
| 76. 0–78. 9 | 11 | 15 | 9 | ..... | ..... |
| 79. 0–81. 9 | 12 | 21 | 6 | ..... | ..... |
| 82. 0–84. 9 | 17 | 31 | 6 | ..... | ..... |
| 85. 0–87. 9 | 12 | 25 | 1 | ..... | ..... |
| 88. 0–90. 9 | 6 | 10 | ..... | ..... | ..... |
| 91. 0–93. 9 | 2 | 7 | ..... | ..... | ..... |
| 94. 0–96. 9 | 1 | 1 | ..... | ..... | ..... |
| Total | 83 | [a] 138 | [a] 26 | 8 | 4 |

[a] Two dwarf eggs of prolate-spheroidal shape, one with some free yolk and one with a small yolk, were laid after the frequency constants and correlation coefficients had been calculated. These eggs are included in Table I, but not in Table II, III, IV, or V.

The size and variation of the different egg parts in dwarf eggs is an interesting but difficult question. It was found possible to separate accurately the parts in a dwarf egg with a small yolk inclosed in yolk

membrane. The method employed was the one in routine use at this laboratory (3). The egg was first weighed; then the egg was broken and the parts separated. The yolk and shell were wiped as dry as possible with filter paper and weighed. The weight of albumen was determined by difference. The weights of the parts were determined for 16 of the small-yolked dwarf eggs. This number is so small that the variation and correlation were studied directly from the ungrouped data. These data are given in Table III.

The frequency constants calculated from the distributions in Table II and from the data in Table III are given in Table IV.

TABLE III.—*Weight of egg and of each of the egg parts for the 16 dwarf eggs on which these data were taken*

| Egg No. | Weight of egg. | Weight of yolk. | Weight of albumen. | Weight of shell. |
|---|---|---|---|---|
| | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 20.00 | 0.85 | 15.50 | 3.65 |
| 2 | 17.00 | 1.00 | 12.00 | 4.00 |
| 3 | 18.40 | 1.85 | 12.75 | 3.80 |
| 4 | 24.50 | 2.19 | 19.09 | 3.22 |
| 5 | 20.00 | 2.50 | 12.50 | 5.00 |
| 6 | 22.50 | 3.55 | 16.70 | 2.25 |
| 7 | 24.85 | 4.37 | 17.10 | 3.38 |
| 8 | 29.00 | 4.50 | 20.00 | 4.50 |
| 9 | 24.75 | 4.53 | 16.17 | 4.05 |
| 10 | 30.00 | 5.00 | 21.25 | 3.75 |
| 11 | 29.00 | 5.50 | 20.00 | 3.50 |
| 12 | 31.00 | 5.50 | 21.00 | 4.50 |
| 13 | 32.50 | 6.50 | 18.50 | 7.50 |
| 14 | 30.33 | 6.50 | 19.83 | 4.00 |
| 15 | 32.00 | 7.00 | 20.25 | 4.75 |
| 16 | 34.00 | 7.00 | 22.00 | 5.00 |
| Mean | 26.24±0.82 | 4.27±0.34 | 17.79±0.53 | 4.18±0.19 |

The variation constants were not calculated for cylindrical eggs either with or without yolk, since the number is so small that these constants would be meaningless. The arithmetical mean in these cases was calculated directly from the data. For the sake of comparison the Table IV also gives the constants determined by Curtis (4) from the 3,180 normal eggs laid by a flock of 22 Barred Plymouth Rock birds during their pullet year, and the constants determined by Pearl and Surface (20) for the 450 eggs laid by an 850-bird flock of Barred Plymouth Rock pullets on February 12, 1908. The constants from the two series agree closely and may be considered a fair measure of the variation in the physical characters of the normal Barred Plymouth Rock egg. Since the second set of constants is based on a group of eggs, no two of which were laid by the same bird, they are theoretically the better measure of a random sample of Barred Plymouth Rock eggs.

TABLE IV.—*Constants of variation in size and shape in the several types of dwarf eggs and in normal eggs*

| Character. | Dwarf eggs. | | | | | All normal first-year eggs from 22-bird flock. | All eggs laid by 850 birds on Feb. 12, 1908. |
|---|---|---|---|---|---|---|---|
| | Prolate-spheroidal shape. | | | Cylindrical shape. | | | |
| | Yolkless. | Some free yolk. | A small yolk. | Yolkless. | Some free yolk. | | |
| Number of eggs | 83 | 138 | 26 | 8 | 4 | 3,180 | 450 |
| Length: | | | | | | | |
| Mean | 35.27±0.37 | 35.84±0.34 | 41.35±0.50 | 41.68 | 31.3 | 55.70±0.03 | 56.32±0.08 |
| Standard deviation | 5.03± .26 | 5.88± .24 | 3.81± .36 | ..... | ..... | 2.41± .02 | 2.39± .05 |
| Coefficient of variation | 14.26± .76 | 16.40± .68 | 9.22± .87 | ..... | ..... | 4.33± .04 | 4.24± .09 |
| Breadth: | | | | | | | |
| Mean | 28.23± .24 | 28.94± .21 | 32.38± .38 | 23.59 | 15.73 | 41.14± .02 | 41.92± .04 |
| Standard deviation | 3.20± .17 | 3.61± .15 | 2.87± .27 | ..... | ..... | 1.41± .01 | 1.38± .03 |
| Coefficient of variation | 11.34± .60 | 12.48± .51 | 8.85± .83 | ..... | ..... | 3.44± .03 | 3.29± .07 |
| Index: | | | | | | | |
| Mean | 80.75± .44 | 81.54± .36 | 78.88± .59 | 57.57 | 48.67 | 73.95± .04 | 74.52± .12 |
| Standard deviation | 5.96± .31 | 6.31± .26 | 4.41± .42 | ..... | ..... | 3.30± .03 | 3.79± .09 |
| Weight: | | | | | | | |
| Mean | 17.11± .43 | 18.35± .41 | 24.81± .81 | 15.24 | 10.63 | 52.92± .06 | 55.26± .15 |
| Standard deviation | 5.86± .31 | 7.14± .29 | 6.13± .57 | ..... | ..... | 5.01± .04 | 4.62± .10 |
| Coefficient of variation | 34.24±1.99 | 38.90±1.80 | 24.70±2.45 | ..... | ..... | 9.46± .08 | 8.36± .19 |
| Weight: | | | | | | | |
| Mean | ..... | ..... | [a]26.24± .82 | ..... | ..... | ..... | ..... |
| Standard deviation | ..... | ..... | [a] 5.29± .63 | ..... | ..... | ..... | ..... |
| Coefficient of variation | ..... | ..... | [a]20.15±2.50 | ..... | ..... | ..... | ..... |
| Yolk weight: | | | | | | | |
| Mean | ..... | ..... | [a] 4.27± .34 | ..... | ..... | 15.77± .02 | ..... |
| Standard deviation | ..... | ..... | [a] 2.01± .24 | ..... | ..... | 1.78± .02 | ..... |
| Coefficient of variation | ..... | ..... | [a]47.12±6.75 | ..... | ..... | 11.31± .11 | ..... |
| Albumen weight: | | | | | | | |
| Mean | ..... | ..... | [a]17.79± .53 | ..... | ..... | 31.55± .05 | ..... |
| Standard deviation | ..... | ..... | [a] 3.15± .37 | ..... | ..... | 3.87± .04 | ..... |
| Coefficient of variation | ..... | ..... | [a]17.69±2.18 | ..... | ..... | 12.27± .11 | ..... |
| Shell weight: | | | | | | | |
| Mean | ..... | ..... | [a] 4.18± .19 | ..... | ..... | 5.12± .01 | ..... |
| Standard deviation | ..... | ..... | [a] 1.10± .13 | ..... | ..... | .71± .01 | ..... |
| Coefficient of variation | ..... | ..... | [a]26.31±3.35 | ..... | ..... | 13.86± .13 | ..... |

[a] Calculated directly from the data (Table III) for the 16 eggs on which the weights of the parts were known.

The flocks which produced the dwarf eggs were largely composed of Barred Plymouth Rock birds from 5 to 17 months of age—that is, the birds were for the most part of the same age and strain as those producing the eggs from which the normal variation constants were calculated. Most of the dwarf eggs were produced by birds of this age and strain. A few were produced by birds of other breeds and a few by older birds. Barred Plymouth Rock birds in their first year produced dwarf eggs which extend over the whole range of size and shape. The slight heterogeneity of the material can not have so materially affected

the variation constants that it is unfair to compare them with the constants for normal Barred Plymouth Rock eggs.

By means of the data given in Tables II, III, and IV it is possible to compare the size, shape, and degree of variability of the several groups of dwarf eggs both among themselves and with normal eggs.

### A.—SIZE RELATION OF DWARF AND NORMAL EGGS

The means given in Table IV show mathematically that all classes of dwarf eggs are of lighter weight and both shorter and narrower than normal eggs. This fact is, of course, obvious from the most casual inspection of dwarf and normal eggs. In comparing the different classes of dwarf eggs with each other it is necessary to keep in mind that the number of cylindrical eggs is so small that the means determined may not represent the true means for this class of eggs. Of the eggs studied, however, the mean prolate-spheroidal, or egg-shaped, egg was decidedly heavier than the mean cylindrical egg. It was also decidedly broader. It can be seen from the means given in the table that the mean weight and the mean breadth for both groups of cylindrical eggs are smaller than the mean for the same character for any group of the prolate-spheroidal eggs. The mean lengths for all cylindrical and all prolate-spheroidal eggs may be compared by calculating from the means in Table IV the weighted mean for each of these shape groups. The mean length for the cylindrical eggs is 38.22 and for the prolate-spheroidal eggs it is 36.23—that is, the cylindrical eggs studied were much lighter in weight, decidedly narrower, but slightly longer than the eggs of the prolate-spheroidal type.

The number of each class of cylindrical eggs is so small that the comparisons of the means for the two classes is of very doubtful meaning. A comparison of the means for the several groups of prolate-spheroidal eggs seems to show that those with small yolks average longer, broader, and heavier than those of the other groups, while the means for the dwarf eggs with some yolk not in membrane (free yolk) are slightly higher than for yolkless dwarf eggs. While the number of dwarf eggs in each group of prolate-spheroidal eggs is larger than in the case of cylindrical dwarf eggs, the actual number is not very large. In order to determine whether or not the above noted differences are greater than those which might arise from errors in sampling, each difference is compared with its probable error. The first section of Table V gives for each physical character measured the deviation in mean with the probable error, and the ratio of the error to the deviation between normal eggs[1]

[1] Since the constants derived from the 450 eggs laid on the same day are measures of an absolutely random sample of Barred Plymouth Rock eggs, these constants are used in calculating the difference between dwarf and normal eggs in the case of length, breadth, index, and weight. Data on the weight of the egg parts were not taken on the 450 egg series. Therefore the only available constants for these characters are those determined from all of the first-year eggs of the small flock.

and small-yolked dwarf eggs, between small-yolked and free-yolked dwarf eggs, and between free-yolked and yolkless dwarf eggs.

TABLE V.—*Deviation between normal eggs and egg-shaped dwarf eggs and between the different classes of egg-shaped dwarf eggs for the mean and coefficient of variation of each measured character*

| Character. | Classes compared. | Difference in mean with probable error. | Difference ÷ Probable error of a difference. |
|---|---|---|---|
| Length | Normal [a]—small-yolked dwarf | 14.97±0.51 | 29.4 |
| Do | Small-yolked—free-yolked dwarf | 5.51± .60 | 9.2 |
| Do | Free-yolked—yolkless dwarf | .57± .50 | 1.1 |
| Breadth | Normal [a]—small-yolked dwarf | 9.54± .38 | 24.5 |
| Do | Small-yolked—free-yolked dwarf | 3.14± .43 | 8.0 |
| Do | Free-yolked—yolkless dwarf | .71± .32 | 2.2 |
| Index | Normal [a]—small-yolked dwarf | −4.36± .60 | 7.3 |
| Do | Small-yolked—free-yolked dwarf | −2.66± .69 | 3.9 |
| Do | Free-yolked—yolkless dwarf | .79± .57 | 1.4 |
| Weight | Normal [a]—small-yolked dwarf | 30.45± .82 | 37.1 |
| Do | Small-yolked—free-yolked dwarf | 6.46± .91 | 7.1 |
| Do | Free-yolked—yolkless dwarf | 1.24± .59 | 2.1 |
| Yolk weight | Normal [b]—small-yolked dwarf [c] | 11.50± .34 | 33.8 |
| Albumen weight | do | 13.76± .53 | 26.0 |
| Shell weight | do | .94± .19 | 4.9 |

| Character. | Classes compared. | Deviation in coefficient of variation with probable error of difference. | Deviation in coefficient of variation ÷ Probable error of difference. |
|---|---|---|---|
| Length | Free-yolked—yolkless dwarf | 2.14±1.02 | 2.1 |
| Do | Yolkless—small-yolked dwarf | 5.04±1.15 | 4.4 |
| Do | Small-yolked dwarf—normal [a] | 4.98± .87 | 5.7 |
| Breadth | Free-yolked—yolkless dwarf | 1.14± .79 | 1.4 |
| Do | Yolkless—small-yolked dwarf | 2.49±1.02 | 2.4 |
| Do | Small-yolked dwarf—normal [a] | 5.56± .83 | 6.7 |
| Weight | Free-yolked—yolkless dwarf | 4.66±2.68 | 1.7 |
| Do | Yolkless—small-yolked dwarf | 9.54±3.16 | 3.0 |
| Do | Small-yolked dwarf—normal [a] | 16.34±2.46 | 6.6 |
| Yolk weight | Small-yolked dwarf [c]—normal [b] | 35.81±6.75 | 5.3 |
| Albumen weight | do | 5.42±2.18 | 2.5 |
| Shell weight | do | 12.45±3.35 | 3.7 |

[a] Calculated from the 450 eggs laid by the flock on a single day.
[b] Calculated from all of the 3,180 eggs laid by a flock of 22 pullets during their first laying year.
[c] Calculated direct from the data for the 16 small-yolked dwarf eggs for which the weights of the parts were known.

It is customary to consider a difference smaller than twice the probable error as probably not significant, a difference between two and three times its probable error as of a doubtful significance, and a difference three or more times the error as certainly or almost certainly significant.

Pearl and Miner (17) have published a table showing, for each value of the ratio of the probable error to the deviation, the probable occurrence in a hundred trials of a deviation as great or greater than the observed, provided chance alone is operating, and also the odds against its occurrence. From this table we see that the odds against a deviation due to chance alone, which is 3.0 times its probable error, is 22.26 to 1. We also see that above 3.0 the increase in odds is very rapid. At 4.0 it is 142.26 to 1; at 5.0 it is 1,350.35 to 1; at 8.0 it is 1,470,588,234 to 1. In the present discussion a deviation less than twice its probable error is considered insignificant. The significance of a deviation between two and three times the probable error is considered doubtful. A deviation between three and four times its probable error is considered probably significant. A deviation four or more times its probable error is considered almost certainly significant, with the understanding that when the odds against the occurrence of a given deviation being due to chance alone are as great or greater than 142.26 to 1, the deviation is almost certainly due to some other cause than error of sampling.

From Table V we see that small-yolked dwarf eggs are significantly smaller than normal eggs and larger than the other classes of dwarf eggs. These significant differences are seen in length, breadth, and weight [1]—that is, the small-yolked egg is nearer the size of a normal egg than are dwarf eggs with little or no yolk. The average length, breadth, and weight are all slightly higher for dwarf eggs which contain some free yolk than for yolkless dwarf eggs. These slight differences may be due to errors in sampling, since in no case is the deviation three times its probable error—that is, although the mean size of the observed dwarf eggs with some free yolk is slightly greater than the mean size of the observed yolkless dwarf eggs, this difference is not certainly significant.

These results are in line with the results from other investigations on the size of eggs. First, Pearl (12) showed that the relation of the weight of the entire egg to the number of yolks contained (zero, one, two, or three) is very accurately described by a parabola. He concluded that, while the size of eggs is not directly proportional to the number of yolks they contain, a definite relation probably exists between the amount of albumen secreted and the amount of yolk present in the duct in a given case. Second, Curtis (5) showed that within the eggs of an individual bird the actual weight of both albumen and shell is higher in triple-yolked than in double-yolked and higher in double-yolked than in single-yolked eggs. The increase in these accessory parts is not, however, proportional to the increase in yolk weight, since the yolk which formed only 24.37 per cent of the normal eggs formed 33.91 per cent of the double-yolked and 35.52 per cent of the triple-yolked eggs. Third, Curtis (4) showed that in the normal eggs of each individual bird there is a significant

[1] The weight of each egg part is also significantly smaller in small-yolked dwarf than in normal eggs.

correlation between the weight of the yolk and the weight of the albumen—that is, the amount of albumen secreted is in part at least dependent on the amount of immediate stimulation due to the quantity of yolk in the duct.

The results recorded for the different classes of dwarf eggs carry these results further. The eggs which contain small-formed yolks are smaller than normal eggs and larger than eggs which contain either little or no yolk. That eggs with a small amount of free yolk are not certainly significantly larger than eggs without yolk is explained by the fact that the two groups were separated strictly on a basis of the presence or absence of yolk. Dwarf eggs which do not contain formed yolks contain as nuclei lumps or drops of free yolk, lumps of hardened secretion, blood clots, or fibers of coagulated albumen. The size of these nuclei vary considerably. A single drop or a very small lump of yolk threw the egg into the class of free-yolked dwarf eggs. Several yolkless dwarf eggs contained nuclei larger than some of the lumps or drops of yolk found in the free-yolked dwarf eggs. In a broad way at least the size of the egg varies with the size of the nucleus—that is, a large dwarf egg contains a considerable amount of yolk or some other large nucleus. A very small one contains a small nucleus. Since the irregular particles can not be accurately measured, the degree of this relationship [1] can not be ascertained.

A comparison of the mean egg size of the several groups of dwarf eggs classified according to yolk content confirms the evidence obtained from a study of normal and multiple-yolked eggs that the amount of yolk (or other nucleus) present in the oviduct is an important factor in determining the amount of albumen secreted in a given case.

### B.—RELATIVE SHAPE OF DWARF AND NORMAL EGGS

Tables IV and V also give data for a study of the comparative shape of the several classes of dwarf and of normal eggs. It has already been noted that there are two distinct shape groups of dwarf eggs: Cylindrical and prolate-spheroidal eggs. A comparison of the mean indices shows that cylindrical dwarf eggs are longer in proportion to their breadth than are normal eggs, while prolate-spheroidal eggs are proportionately shorter than normal eggs. It is also seen that dwarf eggs with small yolks are nearer the shape of normal eggs than are dwarf eggs without formed yolks.

The cause for the distinctly different form in cylindrical and prolate-spheroidal dwarf eggs can not be certainly decided from the material at hand. In several cases of cylindrical dwarf eggs the form of the nucleus was not noted. However, in a few pronounced cases it was noted that

[1] On page 1000 it is shown that in dwarf eggs with formed yolks the yolk weight is highly correlated both with the egg weight and the albumen weight.

the nucleus of coagulated fibers of albumen was drawn out in a line parallel to the long axis of the egg. Further, at one of our routine autopsies there was found in an oviduct a string of albumen 5 or 6 cm. long and not more than 1 cm. in diameter. This was wrapped around a long thread of coagulated albumen fibers which lay parallel to the length of the duct. It seems probable that the form of the stimulating nucleus is one of the factors in determining the shape of the egg. When the stimulus is small in amount and drawn out, the degree of stimulation must be small but the area covered large.

In the prolate-spheroidal eggs the nucleus is usually of globular form—that is, its shape is comparable to the shape of a normal yolk. All the eggs with small-formed yolks were of the prolate-spheroidal type. It has been noted that indices for dwarf eggs with small yolks are higher than those for normal eggs and lower than those for other prolate-spheroidal eggs. The order for the value of index is thus the reverse of the order for the size characters. Later it will be shown that within each group of dwarf eggs the index is negatively correlated with weight. In earlier investigations (3, 5) it has been shown, first, that the indices for multiple-yolked eggs lie below the range of variation for the indices of normal eggs, and, second, that within the normal eggs of an individual the index is negatively correlated with weight. The results from the study of dwarf eggs, therefore, extend the former evidence that the smaller the egg the broader it is in proportion to its length. Two factors may be working together to produce this negative correlation between index and weight. First, the greater the long diameter of the nucleus—be it yolk drop, normal yolk, or two or three yolks in tandem—the longer will be the area of oviduct stimulated at the same time; and, second, when a plastic body is forced (by peristalsis) through an elastic tube the tube will offer less mechanical resistance to the passage of a small than a large body. This mechanical factor is probably of great importance in determining the shape of the egg.

### C.—RELATIVE VARIABILITY OF DWARF AND NORMAL EGGS

Tables IV and V give also the data for comparing the variability of the different classes of prolate-spheroidal dwarf eggs with each other and with normal eggs. Table IV gives for normal eggs and for each class of egg-shaped dwarf eggs the standard deviation for length, breadth, index, and weight, and the coefficient of variation for each of these characters except index.[1] In the case of normal eggs and dwarf eggs with formed yolks it also gives the variation constants for each egg part (yolk, albumen, and shell). A comparison either of standard deviations or of coefficients of variation shows that normal eggs are less variable in each character measured than are the eggs of any class of prolate-spheroidal dwarf eggs. The

[1] Coefficients of variation of percentage characters have no physical significance.

most variable class of dwarf eggs is apparently those which have some free yolk, while the yolkless dwarf eggs are more variable than the small-yolked dwarf eggs—that is, the small-yolked dwarf egg approaches the normal in degree of variability as well as in size and shape. In comparing classes where the absolute difference in size is as great as it is between normal and dwarf eggs the coefficients of variation are more accurate measures of relative variability than are the standard deviations. In order to determine whether or not the apparent difference in degree of variability shown by the several classes is significant, it is necessary to compare these differences with their probable errors. The second section of Table V shows these differences in the size characters with their probable errors and the ratio of each difference to its probable error. From this table we see, first, that normal eggs are significantly less variable than the least variable class of dwarf eggs (small-yolked dwarf eggs) in length, breadth, egg weight, yolk weight, and probably shell weight. The significance of the smaller variation in albumen weight is doubtful. Second, small-yolked dwarf eggs are almost certainly less variable than other dwarf eggs in length and probably also in weight. The significance of the smaller variation in breadth is doubtful. Third, the somewhat greater variation in every size character in the dwarf eggs with free yolk than in the yolkless eggs is not certainly significant—that is, it may be due to errors in sampling.

As previously stated, the coefficient of variation of index, which is a percentage character, has no physical meaning. Since the index equals the percentage that the breadth is of the length, all the indices are measured in the same units and have the same possibilities of variation in range. There is, then, less objection to comparing the standard deviations of such a character; in fact, such a comparison is the only available measure of the relative variability in shape of the several groups. However, too much reliance should not be placed on the figures. The differences in the standard deviations of the indices for the different groups are as follows:

| | |
|---|---|
| Free-yolked—yolkless dwarf | $=0.35 \pm 0.40$ |
| Yolkless—small yolked dwarf | $=1.55 \pm .52$ |
| Small-yolked dwarf—normal | $=.62 \pm .43$ |

The only deviation which can be considered of even probable significance is the difference between yolkless and small-yolked dwarf eggs—that is, normal eggs and small-yolked dwarf eggs are probably less variable in shape than dwarf eggs without a formed yolk.

The relative variability of the size characters within each group is also of some interest. From Table IV it may be seen that the order of variability of the size characters of the egg is the same in normal eggs and in each class of the dwarf eggs. The size characters arranged in the order of their variability from most to least variable are (1) egg weight,

(2) length, and (3) breadth. In order to determine whether or not these apparent differences may be considered significant, the differences with their probable errors and the ratio of each difference to its probable error are given in Table VI.

TABLE VI.—*Difference between the coefficients of variation in the size characters, together with the probable error of difference and the ratio of each difference to its probable error, for normal eggs and for each class of egg-shaped dwarf eggs*

| Class. | Characters compared. | Difference in coefficient of variation, with probable error. | Difference ÷ probable error of difference. |
|---|---|---|---|
| Normal eggs [a] | Egg weight—length | 4.12±0.21 | 19.6 |
| Do | Length—breadth | .95± .11 | 8.6 |
| Small-yolked dwarf eggs. | Egg weight—length | 15.48±2.60 | 6.0 |
| Do | Length—breadth | .37±1.20 | .3 |
| Free-yolked dwarf eggs | Egg weight—length | 22.50±1.92 | 11.7 |
| Do | Length—breadth | 3.92± .85 | 4.6 |
| Yolkless dwarf eggs | Egg weight—length | 19.98±2.13 | 9.4 |
| Do | Length—breadth | 2.92± .97 | 3.0 |

[a] From 450 eggs laid by a flock of Barred Plymouth Rock pullets in a single day.

From Table VI it appears that the order of variability of the characters is probably significant—that is, in both normal and dwarf eggs the size characters may be arranged in the order of their variability as egg weight, length, and breadth. The only case where the difference is less than three times its probable error is between length and breath in "cock eggs" with small yolks. In this case the deviation might have been due to errors of sampling.

In normal eggs it has been shown by Curtis (4) that the weight of the whole egg is less variable than the weight of any part (yolk, albumen, or shell). Of the three parts the shell is the most and the yolk the least variable. The coefficients calculated for 16 dwarf eggs with small yolks do not show the same relative variability of the parts. In these eggs the weight of albumen was less variable than the weight of the whole egg, and the yolk weight instead of being the most constant of the three parts was the most variable. The number of eggs, however, is so small that the probable error of sampling is large. Table VII shows that when the coefficients of variation of the egg parts are arranged in the order of their value, the differences between the two of nearest value is in no case equal to three times the probable error of difference. It is, however, probable that yolk weight is more variable compared to the weight of the other parts and to the whole egg in small-yolked dwarf than in normal eggs.

TABLE VII.—*Relative variability of the whole and the several parts of normal eggs and of dwarf eggs with small yolks*

NORMAL EGGS [a]

| Characters compared. | Difference in coefficient of variation with probable error. | Difference ÷ probable error of difference. |
|---|---|---|
| Shell weight—albumen weight | 1.59±0.17 | 9.4 |
| Albumen weight—yolk weight | .96± .16 | 6.0 |
| Yolk weight—egg weight | 1.85± .14 | 13.2 |
| SMALL-YOLKED DWARF EGGS [b] | | |
| Yolk weight—shell weight | 20.81±7.54 | 2.8 |
| Shell weight—albumen weight | 8.62±4.00 | 2.1 |
| Egg weight—albumen weight | 2.46±3.32 | .7 |

[a] From 3,180 eggs laid by flock of 22 Barred Plymouth Rock pullets.
[b] Coefficients calculated direct from data for the 16 dwarf eggs of known yolk weight.

## IV.—INTERRELATION OF THE DIMENSIONS, SHAPE, AND WEIGHT OF EACH CLASS OF DWARF EGGS COMPARED TO THE SAME RELATIONS IN NORMAL EGGS

We have seen that the dwarf eggs of each group vary greatly in each dimension and in weight and shape. We shall now consider the correlation in the variation of the several characters in prolate-spheroidal[1] dwarf eggs of each class. It will be determined whether a long dwarf egg is broader or narrower than a short one of the same class; whether a large dwarf egg of any class is longer or broader or both longer and broader than a small egg of the same class; whether a large dwarf egg is longer or shorter in proportion to its breadth than a small dwarf of the same group; and whether or not these relations are the same in the several groups of dwarf eggs and in normal eggs. In the case of dwarf eggs with formed yolk the relation between yolk weight and albumen weight is also studied.

The correlations studied then are length with breadth, breadth with weight, length with weight, index with weight, yolk weight with egg weight, and yolk weight with albumen weight. On account of the small number of dwarf eggs of known yolk weight, the correlations involving yolk weight were calculated directly from the data given in Table III. In the case of the other pairs of characters the usual correlation tables were made for each class of dwarf eggs. These are shown as Tables VIII to XIX, inclusive.

[1] Cylindrical eggs appear to show the same relations among themselves as the prolate-spheroidal eggs, but the number is too small to determine the significance of the relationship.

TABLE VIII.—*Correlation between egg breadth and egg length in dwarf eggs with some yolk not in a yolk membrane*

| Egg length (in millimeters). | Egg breadth (in millimeters). | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 18.00–19.99 | 20.00–21.99 | 22.00–23.99 | 24.00–25.99 | 26.00–27.99 | 28.00–29.99 | 30.00–31.99 | 32.00–33.99 | 34.00–35.99 | 36.00–37.99 | Total. |
| 20.00–22.99 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 23.00–25.99 | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 26.00–28.99 | ... | 1 | 3 | 5 | 2 | ... | ... | ... | ... | ... | 11 |
| 29.00–31.99 | ... | 1 | 1 | 7 | 22 | 1 | ... | ... | ... | ... | 32 |
| 32.00–34.99 | ... | ... | 1 | 5 | 9 | 10 | ... | ... | ... | ... | 25 |
| 35.00–37.99 | ... | ... | ... | 1 | 2 | 5 | 7 | 1 | ... | ... | 16 |
| 38.00–40.99 | ... | ... | ... | ... | ... | 8 | 7 | 7 | 1 | ... | 23 |
| 41.00–43.99 | ... | ... | ... | ... | ... | 1 | 4 | 5 | 5 | 1 | 16 |
| 44.00–46.99 | ... | ... | ... | ... | ... | ... | 1 | 1 | 3 | 2 | 7 |
| 47.00–49.99 | ... | ... | ... | ... | ... | ... | 1 | 3 | ... | 1 | 5 |
| 50.00–52.99 | ... | ... | ... | ... | ... | ... | ... | 1 | ... | ... | 1 |
| Total | 1 | 3 | 5 | 18 | 35 | 25 | 20 | 18 | 9 | 4 | 138 |

TABLE IX.—*Correlation between egg breadth and egg length in dwarf eggs without yolk*

| Egg length (in millimeters). | Egg breadth (in millimeters). | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 18.00–19.99 | 20.00–21.99 | 22.00–23.99 | 24.00–25.99 | 26.00–27.99 | 28.00–29.99 | 30.00–31.99 | 32.00–33.99 | 34.00–35.99 | Total. |
| 20.00–22.99 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 23.00–25.99 | ... | 1 | 1 | ... | ... | ... | ... | ... | ... | 2 |
| 26.00–28.99 | ... | 1 | 3 | 1 | ... | ... | ... | ... | ... | 5 |
| 29.00–31.99 | ... | 1 | 3 | 3 | 6 | 1 | ... | ... | ... | 14 |
| 32.00–34.99 | ... | ... | ... | 3 | 2 | 10 | 1 | ... | ... | 16 |
| 35.00–37.99 | ... | ... | ... | ... | 6 | 8 | 9 | ... | ... | 23 |
| 38.00–40.99 | ... | ... | ... | ... | 1 | 3 | 4 | 2 | 1 | 11 |
| 41.00–43.99 | ... | ... | ... | ... | ... | 1 | 4 | 1 | ... | 6 |
| 44.00–46.99 | ... | ... | ... | ... | ... | ... | 2 | 3 | ... | 5 |
| Total | 1 | 3 | 7 | 7 | 15 | 23 | 20 | 6 | 1 | 83 |

TABLE X.—*Correlation between egg breadth and egg length in dwarf eggs with small yolks*

| Egg length (in millimeters). | Egg breadth (in millimeters). | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 26.00–27.99 | 28.00–29.99 | 30.00–31.99 | 32.00–33.99 | 34.00–35.99 | 36.00–37.99 | 38.00–39.99 | Total. |
| 32.00–34.99 | ... | 1 | ... | ... | ... | ... | ... | 1 |
| 35.00–37.99 | ... | 4 | 2 | ... | ... | ... | ... | 6 |
| 38.00–40.99 | ... | ... | 2 | 1 | 1 | ... | ... | 4 |
| 41.00–43.99 | 1 | 1 | 1 | 3 | ... | 1 | ... | 7 |
| 44.00–46.99 | ... | ... | ... | 1 | 6 | ... | ... | 7 |
| 47.00–49.99 | ... | ... | ... | ... | ... | ... | 1 | 1 |
| Total | 1 | 6 | 5 | 5 | 7 | 1 | 1 | 26 |

TABLE XI.—*Correlation between egg weight and egg length in dwarf eggs with some yolk not in a yolk membrane*

| Egg length (in millimeters). | Egg weight (in grams). | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3.00–5.99 | 6.00–8.99 | 9.00–11.99 | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | 30.00–32.99 | 33.00–35.99 | 36.00–38.99 | Total. |
| 20.00–22.99 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 23.00–25.99 | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 26.00–28.99 | ... | 5 | 5 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 11 |
| 29.00–31.99 | ... | 1 | 10 | 20 | 1 | ... | ... | ... | ... | ... | ... | ... | 32 |
| 32.00–34.99 | ... | ... | 2 | 10 | 12 | 1 | ... | ... | ... | ... | ... | ... | 25 |
| 35.00–37.99 | ... | ... | ... | 3 | 4 | 5 | 4 | ... | ... | ... | ... | ... | 16 |
| 38.00–40.99 | ... | ... | ... | ... | 1 | 5 | 8 | 8 | 1 | ... | ... | ... | 23 |
| 41.00–43.99 | ... | ... | ... | ... | ... | 1 | 2 | 7 | 4 | 1 | 1 | ... | 16 |
| 44.00–46.99 | ... | ... | ... | ... | ... | ... | ... | 1 | 2 | ... | 4 | ... | 7 |
| 47.00–49.99 | ... | ... | ... | ... | ... | ... | 1 | ... | 2 | 1 | ... | 1 | 5 |
| 50.00–52.99 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1 | ... | ... | 1 |
| Total | 1 | 7 | 17 | 34 | 18 | 12 | 15 | 16 | 9 | 3 | 5 | 1 | 138 |

TABLE XII.—*Correlation between egg weight and egg length in dwarf eggs without yolk*

| Egg length (in millimeters). | Egg weight (in grams). | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3.00–5.99 | 6.00–8.99 | 9.00–11.99 | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | Total. |
| 20.00–22.99 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 23.00–25.99 | 1 | 1 | ... | ... | ... | ... | ... | ... | ... | 2 |
| 26.00–28.99 | ... | 3 | 2 | ... | ... | ... | ... | ... | ... | 5 |
| 29.00–31.99 | ... | 1 | 7 | 4 | 1 | 1 | ... | ... | ... | 14 |
| 32.00–34.99 | ... | 1 | 1 | 3 | 11 | ... | ... | ... | ... | 16 |
| 35.00–37.99 | ... | ... | ... | 2 | 8 | 10 | 3 | ... | ... | 23 |
| 38.00–40.99 | ... | ... | ... | ... | 1 | 4 | 3 | 2 | 1 | 11 |
| 41.00–43.99 | ... | ... | ... | ... | ... | ... | 3 | 2 | 1 | 6 |
| 44.00–46.99 | ... | ... | ... | ... | ... | ... | ... | 1 | 4 | 5 |
| Total | 2 | 6 | 10 | 9 | 21 | 15 | 9 | 5 | 6 | 83 |

TABLE XIII.—*Correlation between egg weight and egg length in dwarf eggs with small yolks*

| Egg length (in millimeters). | Egg weight (in grams). | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | 30.00–32.99 | 33.00–35.99 | Total. |
| 32.00–34.99 | ... | 1 | ... | ... | ... | ... | ... | ... | 1 |
| 35.00–37.99 | 1 | 3 | 2 | ... | ... | ... | ... | ... | 6 |
| 38.00–40.99 | ... | ... | ... | 2 | 1 | 1 | ... | ... | 4 |
| 41.00–43.99 | ... | 1 | ... | 1 | 2 | 2 | ... | 1 | 7 |
| 44.00–46.99 | ... | ... | ... | ... | ... | 2 | 4 | 1 | 7 |
| 47.00–49.99 | ... | ... | ... | ... | ... | 1 | ... | ... | 1 |
| Total | 1 | 5 | 2 | 3 | 3 | 6 | 4 | 2 | 26 |

TABLE XIV.—*Correlation between egg weight and egg breadth in dwarf eggs with some yolk and not in a yolk membrane*

| Egg breadth (in millimeters). | Egg weight (in grams). | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3.00–5.99 | 6.00–8.99 | 9.00–11.99 | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | 30.00–32.99 | 33.00–35.99 | 36.00–38.99 | Total. |
| 18.00–19.99 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 20.00–21.99 | ... | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 3 |
| 22.00–23.99 | ... | 3 | 1 | ... | 1 | ... | ... | ... | ... | ... | ... | ... | 5 |
| 24.00–25.99 | ... | 1 | 11 | 5 | 1 | ... | ... | ... | ... | ... | ... | ... | 18 |
| 26.00–27.99 | ... | ... | 5 | 27 | 3 | ... | ... | ... | ... | ... | ... | ... | 35 |
| 28.00–29.99 | ... | ... | ... | 2 | 13 | 8 | 2 | ... | ... | ... | ... | ... | 25 |
| 30.00–31.99 | ... | ... | ... | ... | ... | 4 | 11 | 4 | 1 | ... | ... | ... | 20 |
| 32.00–33.99 | ... | ... | ... | ... | ... | ... | 2 | 9 | 5 | 2 | ... | ... | 18 |
| 34.00–35.99 | ... | ... | ... | ... | ... | ... | ... | 2 | 3 | 1 | 3 | ... | 9 |
| 36.00–37.99 | ... | ... | ... | ... | ... | ... | ... | 1 | ... | ... | 2 | 1 | 4 |
| Total | 1 | 7 | 17 | 34 | 18 | 12 | 15 | 16 | 9 | 3 | 5 | 1 | 138 |

TABLE XV.—*Correlation between egg weight and egg breadth in dwarf eggs without yolk*

| Egg breadth (in millimeters). | Egg weight (in grams). | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3.00–5.99 | 6.00–8.99 | 9.00–11.99 | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | Total. |
| 18.00–19.99 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 20.00–21.99 | 1 | 2 | ... | ... | ... | ... | ... | ... | ... | 3 |
| 22.00–23.99 | ... | 3 | 3 | ... | ... | 1 | ... | ... | ... | 7 |
| 24.00–25.99 | ... | 1 | 5 | 1 | ... | ... | ... | ... | ... | 7 |
| 26.00–27.99 | ... | ... | 2 | 7 | 6 | ... | ... | ... | ... | 15 |
| 28.00–29.99 | ... | ... | ... | 1 | 14 | 7 | 1 | ... | ... | 23 |
| 30.00–31.99 | ... | ... | ... | ... | 1 | 7 | 8 | 2 | 2 | 20 |
| 32.00–33.99 | ... | ... | ... | ... | ... | ... | ... | 3 | 3 | 6 |
| 34.00–35.99 | ... | ... | ... | ... | ... | ... | ... | ... | 1 | 1 |
| Total | 2 | 6 | 10 | 9 | 21 | 15 | 9 | 5 | 6 | 83 |

TABLE XVI.—*Correlation between egg weight and egg breadth in dwarf eggs with small yolks*

| Egg breadth (in millimeters). | Egg weight (in grams). | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | 30.00–32.99 | 33.00–35.99 | Total. |
| 26.00–27.99 | ... | 1 | ... | ... | ... | ... | ... | ... | 1 |
| 28.00–29.99 | ... | 4 | 1 | 1 | ... | ... | ... | ... | 6 |
| 30.00–31.99 | 1 | ... | 1 | 2 | 1 | ... | ... | ... | 5 |
| 32.00–33.99 | ... | ... | ... | ... | 2 | 3 | ... | ... | 5 |
| 34.00–35.99 | ... | ... | ... | ... | ... | 2 | 4 | 1 | 7 |
| 36.00–37.99 | ... | ... | ... | ... | ... | ... | ... | 1 | 1 |
| 38.00–39.99 | ... | ... | ... | ... | ... | 1 | ... | ... | 1 |
| Total | 1 | 5 | 2 | 3 | 3 | 6 | 4 | 2 | 26 |

TABLE XVII.—*Correlation between egg weight and egg index in dwarf eggs with some yolk not in a yolk membrane*

| Egg index (in percentage). | Egg weight (in grams). | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3.00–5.99 | 6.00–8.99 | 9.00–11.99 | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | 30.00–32.99 | 33.00–35.99 | 36.00–38.99 | Total. |
| 64.00–66.99 | ... | ... | ... | ... | ... | ... | ... | ... | 1 | 2 | ... | ... | 3 |
| 67.00–69.99 | ... | ... | ... | 1 | ... | 1 | 1 | 1 | 1 | ... | ... | ... | 5 |
| 76.00–72.99 | ... | 1 | ... | ... | 2 | 1 | 1 | ... | 1 | ... | ... | ... | 6 |
| 73.00–75.99 | ... | ... | 1 | 3 | 2 | 2 | 3 | 2 | 1 | ... | ... | ... | 14 |
| 76.00–78.99 | ... | ... | 2 | 2 | 1 | 2 | 1 | 3 | 2 | ... | 1 | 1 | 15 |
| 79.00–81.99 | ... | 2 | ... | 3 | 3 | 2 | 5 | 3 | 1 | 1 | 1 | ... | 21 |
| 82.00–84.99 | ... | 1 | 3 | 10 | 5 | 3 | 2 | 3 | 1 | ... | 3 | ... | 31 |
| 85.00–87.99 | ... | ... | 8 | 7 | 3 | ... | 2 | 4 | 1 | ... | ... | ... | 25 |
| 88.00–90.99 | ... | 2 | 2 | 3 | 2 | 1 | ... | ... | ... | ... | ... | ... | 10 |
| 91.00–93.99 | 1 | 1 | 1 | 4 | ... | ... | ... | ... | ... | ... | ... | ... | 7 |
| 94.00–96.99 | ... | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| Total | 1 | 7 | 17 | 34 | 18 | 12 | 15 | 16 | 9 | 3 | 5 | 1 | 138 |

TABLE XVIII.—*Correlation between egg weight and egg index in dwarf eggs without yolk*

| Egg index (in percentage). | Egg weight (in grams). | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3.00–5.99 | 6.00–8.99 | 9.00–11.99 | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | Total. |
| 64.00–66.99 | ... | ... | ... | ... | ... | ... | ... | ... | 1 | 1 |
| 67.00–69.99 | ... | ... | ... | ... | ... | ... | ... | ... | 2 | 2 |
| 70.00–72.99 | ... | ... | 1 | ... | 1 | ... | 1 | ... | ... | 3 |
| 73.00–75.99 | ... | 1 | 1 | 2 | 2 | 4 | 2 | 3 | 1 | 16 |
| 76.00–78.99 | 1 | 1 | 1 | ... | 4 | 1 | 2 | ... | 1 | 11 |
| 79.00–81.99 | ... | 1 | 1 | 2 | 3 | 4 | ... | 1 | ... | 12 |
| 82.00–84.99 | ... | 1 | 2 | 2 | 4 | 6 | 1 | 1 | ... | 17 |
| 85.00–87.99 | ... | 1 | 1 | 2 | 4 | ... | 3 | ... | 1 | 12 |
| 88.00–90.99 | 1 | ... | 3 | 1 | 1 | ... | ... | ... | ... | 6 |
| 91.00–93.99 | ... | 1 | ... | ... | 1 | ... | ... | ... | ... | 2 |
| 94.00–96.99 | ... | ... | ... | ... | 1 | ... | ... | ... | ... | 1 |
| Total | 2 | 6 | 10 | 9 | 21 | 15 | 9 | 5 | 6 | 83 |

TABLE XIX.—*Correlation between egg weight and egg index in dwarf eggs with small yolks*

| Egg index (in percentage). | Egg weight (in grams). | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 12.00–14.99 | 15.00–17.99 | 18.00–20.99 | 21.00–23.99 | 24.00–26.99 | 27.00–29.99 | 30.00–32.99 | 33.00–35.99 | Total. |
| 65.00–67.99 | ... | 1 | ... | 1 | ... | ... | ... | ... | 2 |
| 68.00–70.99 | ... | ... | ... | ... | ... | ... | ... | ... | 0 |
| 71.00–73.99 | ... | ... | ... | ... | ... | 1 | ... | ... | 1 |
| 74.00–76.99 | ... | ... | ... | ... | 1 | ... | 2 | ... | 3 |
| 77.00–79.99 | ... | ... | 1 | 1 | 1 | 4 | 2 | 1 | 10 |
| 80.00–82.99 | ... | 3 | 1 | 1 | ... | ... | ... | ... | 5 |
| 83.00–85.99 | 1 | 1 | ... | ... | 1 | 1 | ... | 1 | 5 |
| Total | 1 | 5 | 2 | 3 | 3 | 6 | 4 | 2 | 26 |

Table XX shows the correlation coefficients deduced from Tables VIII to XIX (or in the case of yolk weight from the data) with their probable errors, and also similar coefficients for normal eggs. The correlations are all calculated from the usual Bravais formula

$$r = \frac{S(xy)}{N\sigma_1\sigma_2}$$

where $N$ = number of eggs; $x$ and $y$ are the deviations from the means and $\sigma_1$ and $\sigma_2$ the standard deviations of the two variables.

TABLE XX.—*Correlation coefficients for the size and shape characters of the dwarf and normal eggs*

| Kind of eggs. | Number of eggs. | Correlation coefficients. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Length and breadth. | Length and weight. | Breadth and weight. | Index and weight. | Yolk weight and egg weight. | Yolk weight and albumen weight. |
| Free-yolked dwarf | 138 | 0.874±0.013 | 0.933±0.007 | 0.950±0.006 | −0.408±0.048 | ..... | ..... |
| Yolkless dwarf | 83 | .844± .021 | .924± .011 | .919± .011 | − .368± .064 | ..... | ..... |
| Small-yolked dwarf | 26 | .759± .056 | .840± .039 | .883± .029 | − .092± .131 | [a]0.940±0.020 | [a]0.789±0.064 |
| Normal (mean of individual coefficients for 22 birds) | 3,180 | .378 | .753 | .830 | − .185 | .818 | .566 |
| Normal (eggs laid by flock on a single day) | 450 | .084± .032 | .580± .021 | .836± .010 | .085± .032 | ..... | ..... |

[a] These coefficients were calculated directly from the data given in Table III.

Table XXI gives the differences between various of these correlation coefficients, the probable error of the differences, and the ratio of each difference to its probable error.

TABLE XXI.—*Deviation in correlation coefficients, the probable error of the differences, and the ratio of each difference to its probable error*

| Class of eggs. | Pairs of characters, the correlation coefficients of which are compared. | Difference in correlation coefficients with probable error. | Difference ÷ probable error of difference. |
|---|---|---|---|
| Free-yolked dwarf | Breadth with weight—length with weight | 0.017±0.009 | 2.0 |
| Do | Length with weight—length with breadth | .059± .015 | 3.9 |
| Do | Length with breadth—index with weight | .466± .050 | 9.3 |
| Yolkless dwarf | Breadth with weight—length with weight | —.005± 016 | .3 |
| Do | Length with weight—length with breadth | .080± .024 | 3.3 |
| Do | Length with breadth—index with weight | .476± .067 | 7.1 |
| Small-yolked dwarf | Breadth with weight—length with weight | .043± .049 | .9 |
| Do | Length with weight—length with breadth | .081± .068 | 1.2 |
| Do | Length with breadth—index with weight | .667± .142 | 4.7 |
| Normal | Breadth with weight—length with weight | .256± .023 | 11.1 |
| Do | Length with weight—length with breadth | .496± .038 | 13.1 |
| Do | Length with breadth—index with weight | —.001± .045 | .02 |

| Pairs of characters correlated. | Classes compared. | Difference in correlation coefficients with probable error. | Difference ÷ probable error of difference. |
|---|---|---|---|
| Length with breadth | Free-yolked—yolkless | 0.030±0.025 | 1.2 |
| Do | Yolkless—small-yolked | .085± .060 | 1.4 |
| Do | Small-yolked—normal | .675± .065 | 10.4 |
| Length with weight | Free-yolked—yolkless | .009± .013 | .7 |
| Do | Yolkless—small-yolked | .084± .041 | 2.1 |
| Do | Small-yolked—normal | .260± .044 | 5.9 |
| Breadth with weight | Free-yolked—yolkless | .031± .013 | 2.4 |
| Do | Yolkless—small-yolked | .036± .031 | 1.2 |
| Do | Small-yolked—normal | .047± .031 | 1.5 |
| Index with weight | Free-yolked—yolkless | .040± .080 | 2.5 |
| Do | Yolkless—small-yolked | .276± .146 | 1.9 |
| Do | Small-yolked—normal | .007± .135 | .05 |
| Yolk weight with egg weight. | do | .122 | ..... |
| Yolk weight with albumen weight. | do | .223 | ..... |

From Table XX the following points may be noted:

1. In each class of dwarf eggs the correlation between the two dimensions is positive and is certainly significant—that is, a broad dwarf egg is also long, and vice versa. The shape of the egg is no doubt determined by the action of the longitudinal and circular muscle fibers of the oviduct walls, especially during the formation of the egg membrane and shell. The egg is a fluid body which tends to take a spherical shape when not under pressure. At the time an egg receives its membrane and shell a normal egg or almost any dwarf egg is larger than the normal diameter of the oviduct. It is therefore under pressure which tends to elongate it in the direction of the long axis of the duct. The degree of pressure and, hence, the resulting degree of elongation will depend on (a) the size of the egg compared to the diameter of a cross section of the duct, and

(b) the relative tonus of the two sets of muscle fibers of the oviduct wall. A decrease in the tonus of the circular fibers, or an increase in that of the longitudinal fibers, or both, may counterbalance the increase in pressure due to increase in the diameter of the egg. There is no a priori reason for assuming a correlation between breadth and length; in fact, this correlation was not significant in the random sample of normal eggs studied by Pearl and Surface (20). From this they concluded that the two sets of muscles are to a large extent independent in their action. On the other hand, Curtis (4) found that within the normal eggs of an individual there is usually [1] a significant correlation between length and breadth—that is, the size of the active oviduct and relative tonus of the two sets of muscle fibers in the oviduct wall are apparently usually relatively stable in an individual, and an increase in the breadth of the egg is correlated with an increase in the length. The fact that the correlation between length and breadth is significantly higher (Table XXI) for dwarf eggs than for normal eggs may indicate that in these small eggs there is little or no differential stimulus on the muscle fibers of the oviduct wall, but that there is such a stimulus when the egg is larger.

2. Length and breadth are both highly correlated with weight—that is, a heavy egg is both broad and long. These relations are also true for normal eggs. The random sample of eggs studied by Pearl and Surface (20) showed a correlation between breadth and weight which was significantly higher than the correlation between length and weight. The individual birds studied by Curtis (4) showed a great variation in the relative degree of correlation of the two dimensions with the weight. Half the flock showed a correlation for breadth and weight significantly higher than for length and weight. Two birds showed a higher length-weight correlation. For one-third of the flock the difference was insignificant. There is no significant difference between breadth-weight and length-weight correlation in any class of dwarf eggs. (See Table XXI.)

3. The index-weight correlations are negative, and they are significant for dwarf eggs with little or no yolk—that is, for those two groups of small dwarf eggs the larger the egg the longer it is in proportion to its breadth. In the study of the normal eggs of individual birds Curtis (4) found that there was a low negative correlation between index and weight which was significant for one-half of the individuals studied. This tendency toward a negative correlation between index and weight in dwarf and normal eggs is in line with the fact that the mean index of the several groups of dwarf eggs, normal eggs, and multiple-yolked eggs varies in the opposite direction from the mean egg weight of each group—that is, the larger the egg the lower the index. The bearing of this fact has already been discussed.

[1] In 18 out of 22 individuals studied.

4. The correlation between yolk weight and egg weight in dwarf eggs with small yolks is very high. Since the yolk weight forms part of the egg weight, we will confine our discussion to the correlation between yolk weight and albumen weight. This correlation is also very high. It is in fact higher than the average correlation between yolk weight and albumen weight within the normal eggs of a single individual. This high correlation between yolk weight and albumen weight in dwarf eggs with small yolks adds to the evidence already presented that the amount of yolk present in the duct is an important factor in determining the amount of albumen secreted, and thus both directly and indirectly influences the size of the egg.

## V.—FREQUENCY OF THE OCCURRENCE OF DWARF EGGS COMPARED TO NORMAL EGGS AND OF DWARF EGG PRODUCERS COMPARED TO BIRDS WHICH DO NOT LAY DWARF EGGS.

As previously stated, the period covered by this investigation extends from February 1, 1908, to February 1, 1916. During this period it has been the practice to make up the flock in September or early October. A few of the birds of the previous flock are saved for specific experiments and the rest killed or sold. The pullets are put in the houses at this time. The 298 dwarf eggs collected were thus produced by nine different flocks of birds. The number laid by each flock is given below:

| | Number of dwarf eggs. |
|---|---|
| Feb. 1, 1908, to Aug. 31, 1908 | 16 |
| Sept. 1, 1908, to Aug. 31, 1909 | 20 |
| Sept. 1, 1909, to Aug. 31, 1910 | 34 |
| Sept. 1, 1910, to Aug. 31, 1911 | 43 |
| Sept. 1, 1911, to Aug. 31, 1912 | 59 |
| Sept. 1, 1912, to Aug. 31, 1913 | 17 |
| Sept. 1, 1913, to Aug. 31, 1914 | 27 |
| Sept. 1, 1914, to Aug. 31, 1915 | 72 |
| Sept. 1, 1915, to Feb. 1, 1916 | 10 |
| Feb. 1, 1908, to Feb. 1, 1916 | 298 |

The first and last years are, of course, incomplete. The fluctuations between the other years are no doubt due largely to three causes. First, the size of the flock differs somewhat from year to year. Second, the average annual egg production fluctuates with changes in the proportion of low and high laying strains which compose the successive flocks—for example, the 1914–15 flock contained 55 less birds than the 1911–12 flock, and at the same time produced 25,374 more eggs, so that although it produced 22 per cent more dwarf eggs, the proportion of these eggs to the normal eggs was smaller. Third, as will be discussed later, certain birds suffer disturbances of physiology which cause them to produce a number of dwarf eggs. Such birds do not occur every year; in fact, an unusual proportion of the known cases occurred during the two years of highest dwarf-egg pro-

duction—that is, 1911–12 and 1914–15. During any year a few dwarf eggs may have escaped collection by being broken in the nest or laid on the floor and lost in the litter. This loss can not have been large at any time. However, in order to avoid the possibility of an unequal loss during the several years, the two years of highest dwarf-egg production were selected for a comparison as to the frequency of dwarf and normal eggs.

The frequency of the occurrence of dwarf eggs compared to normal eggs may be determined by calculating the percentage of all the eggs produced which are dwarf. For convenience this percentage may be multiplied by 100. This number represents the number of dwarfs in 10,000 eggs. This percentage was calculated for each of the 12 months of the two years taken both separately and combined. These data are given in Table XXII.

TABLE XXII.—*Total egg production, dwarf-egg production, and number of dwarf eggs per 10,000 eggs for each month of the years 1911–12 and 1914–15 both separately and combined, also for the two years combined the percentage of all the eggs and of all of the dwarf eggs which were produced during each calendar month*

| Month. | 1911–12 | | | 1914–15 | | | 1911–12 and 1914–15 combined. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total eggs. | Dwarf eggs. | Dwarf eggs per 10,000 eggs. | Total eggs. | Dwarf eggs. | Dwarf eggs per 10,000 eggs. | Total eggs. | Dwarf eggs. | Dwarf eggs per 10,000 eggs. | Percentage of total number of eggs produced during month. | Percentage of total number of dwarf eggs produced during month. |
| September..... | 1,870 | 2 | 10.7 | 694 | 1 | 14.4 | 2,564 | 3 | 11.7 | 1.69 | 2.29 |
| October..... | 2,940 | 1 | 3.4 | 2,589 | 0 | .0 | 5,529 | 1 | 1.8 | 3.64 | .76 |
| November..... | 2,713 | 2 | 7.4 | 3,771 | 6 | 15.9 | 6,484 | 8 | 12.3 | 4.27 | 6.11 |
| December..... | 4,501 | 2 | 4.4 | 5,416 | 3 | 5.5 | 9,917 | 5 | 5.0 | 6.54 | 3.82 |
| January..... | 4,429 | 2 | 4.5 | 7,131 | 4 | 5.6 | 11,560 | 6 | 5.2 | 7.62 | 4.58 |
| February..... | 4,375 | 0 | .0 | 8,409 | 3 | 3.5 | 12,784 | 3 | 2.3 | 8.43 | 2.29 |
| March..... | 9,407 | 7 | 7.4 | 9,728 | 5 | 5.1 | 19,135 | 12 | 6.3 | 12.61 | 9.16 |
| April..... | 7,886 | 7 | 8.9 | 11,406 | 8 | 7.0 | 19,292 | 15 | 7.8 | 12.71 | 11.45 |
| May..... | 7,738 | 10 | 12.9 | 11,051 | 7 | 6.3 | 18,789 | 17 | 9.0 | 12.38 | 12.98 |
| June..... | 7,121 | 17 | 23.9 | 10,080 | 10 | 9.9 | 17,201 | 27 | 15.7 | 11.34 | 20.61 |
| July..... | 5,539 | 2 | 3.6 | 9,618 | 20 | 20.7 | 15,157 | 22 | 14.5 | 9.99 | 16.79 |
| August..... | 4,657 | 7 | 15.0 | 8,667 | 5 | 5.7 | 13 324 | 12 | 9.0 | 8.78 | 9.16 |
| Total.... | 63,176 | 59 | 9.3 | 88,560 | 72 | 8.1 | 151,736 | 131 | 8.6 | 100.0 | 100.0 |

The last line in Table XXII shows the total number of eggs, the total number of dwarf eggs, and the number of dwarf eggs per 10,000 for each of the two years, and for the two years combined. From these data it is seen that during the year 1911–12 the flock produced 59 dwarf eggs out of a total of 63,176, or 9.3 dwarf eggs in 10,000—that is, 1 dwarf egg in each 1,071 eggs. In 1914–15 the flock produced 72 dwarf eggs in a total of 88,560 eggs—that is, 8.1 dwarf eggs in 10,000, or 1 dwarf to 1,230 eggs. If the data for the two years are combined, there were produced 131 dwarf eggs in 151,736 eggs—that is, during the two years of maxi-

mum dwarf-egg production the proportion of dwarf to normal eggs was 8.6 dwarf eggs in 10,000, or 1 dwarf egg in 1,158 eggs. Warner and Kirkpatrick (26) show that during two laying contests at Storrs, Conn., 199,137 eggs were produced, of which 103 weighed less than 0.09 pound (40.82 gm.). From these figures we see that they obtained 5.2 dwarf eggs per 10,000, or 1 dwarf in 1,933 eggs.

The nine flocks which laid the dwarf eggs considered in this investigation contained approximately 4,800 different individual birds. Not all of these birds had an equal opportunity to lay a dwarf egg, for while a large majority of them were kept until, and only until, the end of their pullet year, a number died at varying ages and a number were kept for more than one year. Also the records for 1907–8 and 1915–16 are incomplete. We may, however, arrive at an approximate estimate of the proportion of birds which lay one or more eggs by neglecting these discrepancies and considering that each of the 4,800 individuals had an equal opportunity to produce dwarf eggs.

The 251 dwarf eggs of known origin were produced by 200 different individuals. There were 47 eggs laid by birds whose number was not known. Most of these were floor eggs. In a very few cases the poultryman neglected to record the number of the bird on the egg at collection time, and in a very few others the trap-nest record of the bird laying the dwarf egg was lost through some other slip. Since most of the dwarf eggs of known origin were produced each by a different individual, we shall arrive at the fairest estimation of the number of birds which produce dwarf eggs by considering that each of these 47 was laid by a different individual, and by one which had not produced one of the dwarf eggs of known origin—that is, we may consider that the 298 eggs collected were produced by 247 individuals. From the above considerations it appears that during the last eight years at the plant of the Maine Station 247 out of 4,800 birds, or 5.15 per cent, produced at least one dwarf egg.

By means of the data given by Warner and Kirkpatrick (26) we may also approximate the relative number of dwarf-egg producers among the birds in the third and fourth laying contest at Storrs, Conn. These birds also did not all have an equal chance, since the data were worked up after 7 of the 12 months of the fourth contest. During these contests 85 out of 1,820 birds, or 4.67 per cent, laid one or more dwarf eggs. If the data had been digested after the fourth contest had been completed, it is quite probable that a few more birds would have laid dwarf eggs—that is, the percentage given may be too low.

The close agreement of the two approximations indicates that about 5 per cent of the birds in an average flock will produce at least one dwarf egg.

## VI.—SEASONAL FREQUENCY OF DWARF EGGS COMPARED TO NORMAL EGGS

Dwarf eggs are frequently found by poultrymen during the spring and early summer and somewhat less frequently at other seasons. During the eight years that these eggs have been collected at the plant of the Maine Station they have occurred during every one of the 12 months. However, 70.8 per cent of them were laid during the five months from March 1 to July 31. During some years more than 80 per cent were produced during these months. Table XXIII gives the number of dwarf eggs produced each month for each of the eight years. It gives also the percentage of all of the dwarf eggs which were produced during each calendar month and the monthly percentage of the annual egg yield as determined by Pearl and Surface (19) for the years 1899 to 1907.

TABLE XXIII.—*Number of dwarf eggs recorded each month from February 1, 1908, to February 1, 1916, and the percentage of the total number of dwarf (1908–1916) and normal (1899–1907) produced during each calendar month*

| Year. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May. | June. | July. | Aug. | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1907 and 1908.. | ..... | ..... | .... | .... | .... | 1 | 1 | 5 | 5 | 4 | 0 | 0 | [a] 16 |
| 1908 and 1909.. | 0 | 0 | 0 | 0 | 0 | 3 | 7 | 5 | 1 | 3 | 0 | 1 | 20 |
| 1909 and 1910.. | 2 | 1 | 1 | 0 | 0 | 3 | 7 | 5 | 5 | 0 | 10 | 0 | 34 |
| 1910 and 1911.. | 0 | 1 | 2 | 3 | 1 | 2 | 3 | 3 | 12 | 6 | 4 | 6 | 43 |
| 1911 and 1912.. | 2 | 1 | 2 | 2 | 2 | 0 | 7 | 7 | 10 | 17 | 2 | 7 | 59 |
| 1912 and 1913.. | 1 | 1 | 1 | 1 | 3 | 0 | 1 | 0 | 7 | 1 | 1 | 0 | 17 |
| 1913 and 1914.. | 0 | 3 | 0 | 0 | 2 | 0 | 4 | 8 | 4 | 5 | 1 | 0 | 27 |
| 1914 and 1915.. | 1 | 0 | 6 | 3 | 4 | 3 | 5 | 8 | 7 | 10 | 20 | 5 | 72 |
| 1915 and 1916.. | 0 | 5 | 2 | 2 | [b] 1 | .... | ..... | ..... | ..... | ..... | ..... | .... | [a] 10 |
| Total.... | 6 | 12 | 14 | 11 | 13 | 12 | 35 | 41 | 51 | 46 | 38 | 19 | 298 |
| Percentage of total number produced during month.. | 2. 01 | 4. 03 | 4. 71 | 3. 69 | 4. 36 | 4. 03 | 11. 74 | 13. 76 | 17. 11 | 15. 44 | 12. 76 | 6. 36 | 100 |
| Percentage of total annual yield of normal eggs produced during month (1899–1907) . | [c] 6. 36 | [c] 4. 27 | 3. 59 | 6. 91 | 9. 08 | 8. 44 | 12. 50 | 12. 30 | 10. 80 | 9. 67 | 8. 44 | 7. 64 | 100 |

[a] Years incomplete.
[b] Calculations in earlier parts of paper were completed before this egg was laid.
[c] These should follow August, as they are for the end and not the beginning of the year.

The more frequent occurrence of dwarf eggs during the spring and summer is seen either in the record for each year or in the sums at the foot of Table XXIII. It must be kept in mind that this is the natural breeding season of the fowl and that the total number of eggs laid during these months is greater than during the other months of the year. Whether or not the number of dwarf eggs in the breeding season is greater than is to be

expected if they occur in a given ratio to normal eggs can only be decided by a comparison of the production of dwarf eggs with the normal-egg production.

The monthly distribution of normal-egg production has been investigated thoroughly in the Maine Station flock by Pearl and Surface (19). Their investigations cover the eight years preceding the beginning of the present study. They summarized their data for the whole period by obtaining the percentage of the total yearly egg production which occurred during each month. This egg-production polygon may be used as a basis for a rough comparison between the relative seasonal frequency of dwarf and normal eggs. Figure 1 shows this egg-production polygon and a similar polygon showing for the eight years of the present investigation

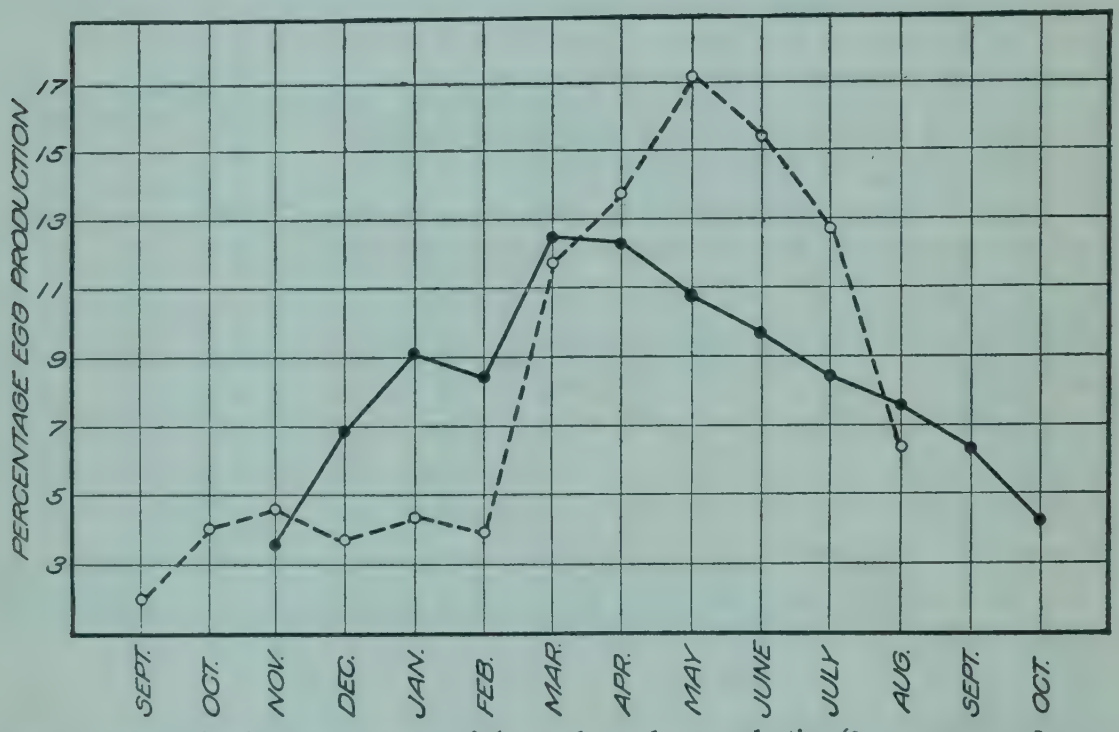


FIG. 1.—Diagram showing the percentage of the yearly total egg production (8-year average, 1899–1907) and the percentage of the total dwarf-egg production (8-year average, 1908–1916) which occurred during each month. Solid line=percentage of annual egg production. Dash line=percentage of annual dwarf-egg production.

the percentage of all of the dwarf eggs which were produced during each month. The data are given in the last two lines of Table XXIII. It will be noted that the two polygons do not begin or end with the same month. The reason for this is that the first set of data was collected for September and October after the birds were a year old, while, as already stated, during the period covered by the second investigation the data from September 1 to September 1 represent more nearly the data on a single group of birds.

From the diagram it is seen, as would be expected on the theory of chance, that during the months of heaviest normal-egg production more dwarf eggs are produced than at other seasons. Yet it is also seen that the two curves are by no means parallel. The egg-production curve rises

gradually through the fall and winter to its spring maximum and then drops away even more gradually. The dwarf-egg production curve does not rise during the fall and winter, but rises very abruptly during the spring to its maximum, which is three months later than the maximum for the normal-egg curve. It remains relatively higher than the normal curve through the early summer.

Since the data for the two polygons are derived from entirely different birds, it is desirable to pursue the investigations further and compare the

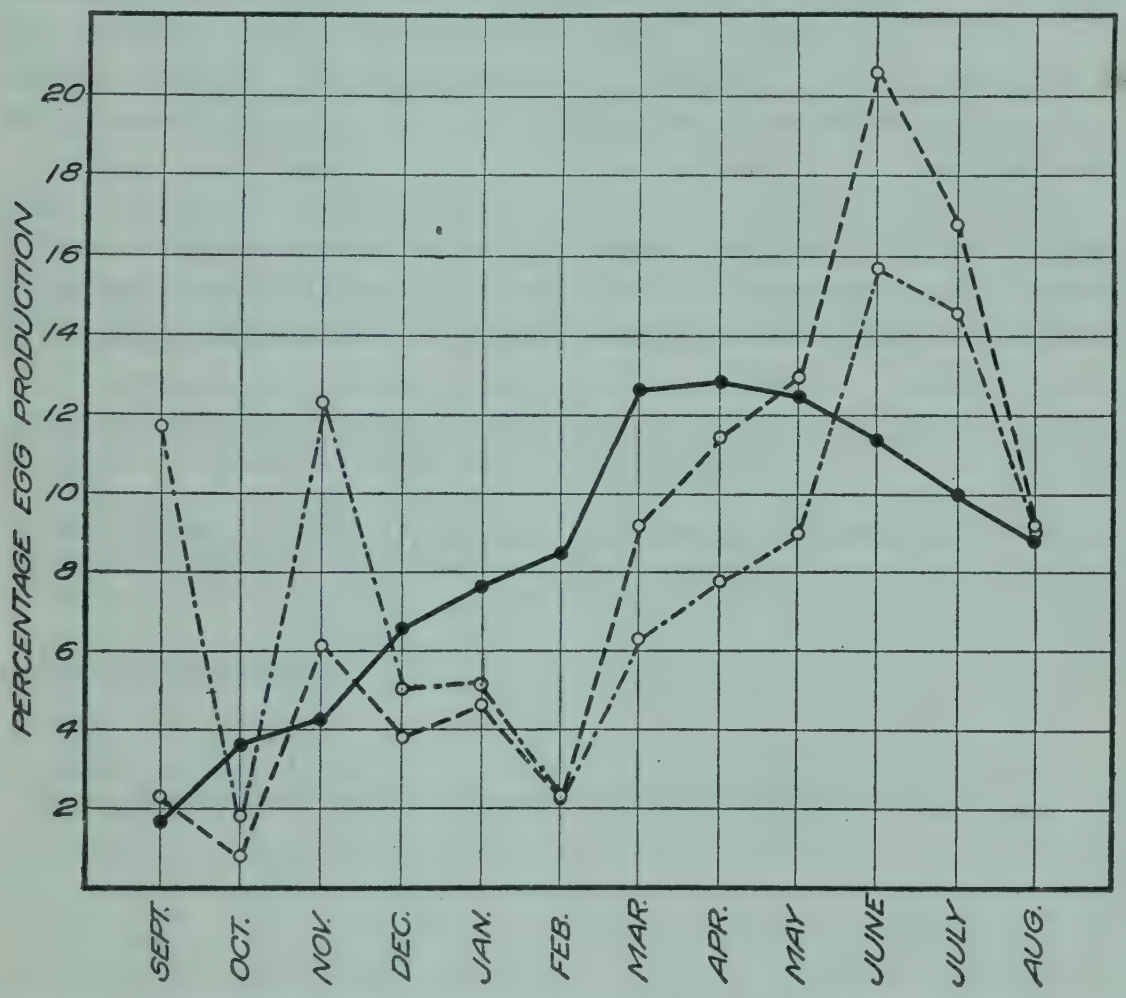


FIG. 2.—Diagram showing for the years 1911–12 and 1914–15 combined the percentage of the yearly total egg production and dwarf-egg production which occurred during each month and 100 times the percentage of the eggs produced each month which were dwarf. Solid line=percentage of total yearly egg production per month. Dash line=percentage of total yearly dwarf-egg production per month. Dot-dash line=the percentage (× 100) of dwarf eggs produced during the month.

number of dwarf eggs and the number of normal eggs produced by the same birds. The two years of maximum dwarf-egg production, 1911–12 and 1914–15, were selected for this study. The data for this study are given in Table XXII, which shows the total egg production, the dwarf-egg production, and the number of dwarf eggs per 10,000 eggs for each month of the two years. The last five columns of the table give the data for the two years combined. The summary data given in the last three columns are shown graphically in figure 2.

An examination of the diagram or of the data given in Table XXII shows that not only is the actual number of dwarf eggs smallest during the winter but that the number of dwarf eggs per 10,000 is also smallest. The irregular fluctuations of the fall are due to the fact that three of the abnormal birds already referred to laid during these months. The small number of normal eggs produced at this season gives great weight to these dwarf eggs in calculating the number of dwarf eggs per 10,000. Both the actual number of dwarf eggs and number per 10,000 increases through the spring, reaching its maximum in early summer some months later than the maximum for normal-egg production. It is thus shown that the dwarf-egg production is actually highest and also highest in proportion to the normal-egg production during the spring and early summer.

It thus seems probable that the disturbances in physiology which result in the production of dwarf eggs become more frequent with the onset of the natural breeding season and continue to increase in frequency during this season. The probable nature of these disturbances will be discussed later.

## VII.—DWARF EGG PRODUCTION BY BIRDS WITH NORMAL AND WITH PATHOLOGICAL OVIDUCTS

The production of a dwarf egg is usually an isolated phenomenon—that is, a bird usually produces only one such egg. This fact, which has already been noted, is easily seen from Table XXIV.

TABLE XXIV.—*Number of dwarf eggs laid by each bird which produced one or more such eggs*

| Number of dwarf eggs laid by a bird. | Number of birds. | Percentage of birds. | Number of eggs. |
|---|---|---|---|
| 1 | 178 | 89.0 | 178 |
| 2 | 15 | 7.5 | 30 |
| 3 | 3 | 1.5 | 9 |
| 4 | 1 | 0.5 | 4 |
| 5 | 1 | 0.5 | 5 |
| 8 | 1 | 0.5 | 8 |
| 17 | 1 | 0.5 | 17 |
| Total | 200 | 100.0 | 251 |
| Number of dwarf eggs laid by birds whose number was not known—that is, mostly floor eggs | ..... | ..... | 47 |
| Total | ..... | ..... | 298 |

From Table XXIV we see that of the 200 birds which produced one or more dwarf eggs, 178, or 89.0 per cent, produced only one; 15, or 7.5 per cent, produced two; and only 7, or 3.5 per cent, more than two. The figures given by Warner and Kirkpatrick (26) for the birds in the Connecticut Station laying contest show an even larger percentage (94.11 per cent) of the dwarf-egg producers which lay only one dwarf egg. One

bird laid 14 dwarf eggs and no normal eggs. Each of the four others (4.71 per cent) laid two. It is thus apparent that the production of dwarf eggs is not usually an evidence of a permanent abnormality or derangement of the reproductive organs. This view is strengthened by a study of the egg records for the birds which produced dwarf eggs. In almost all cases these birds have a normal egg record. The dwarf egg is preceded and followed by normal eggs quite as though it was a normal egg. Autopsies were performed on several such birds, some immediately after the production of the dwarf egg. The sex organs were morphologically normal. There were, however, 11 of the 200 which showed evidence of a permanent disturbance, since few or no normal eggs were produced after the dwarf egg or eggs. In most of these cases the bird made nesting records. It has been shown by the authors (6,13) that "nesting records are, in the great majority of cases, at least, associated with ovulation into the body cavity or the backing into it of partly or fully formed eggs." Furthermore, autopsies were made on 5 of the 11 cases and all of these showed pathological conditions of the oviduct which would interfere with the passage of the egg but which did not entirely close the duct. These cases will be discussed in detail later. The point with which we are at present concerned is that the records for only 11 (5.5 per cent) of the 200 birds showed evidence of a permanent disturbance of the egg-forming processes. It is then evident that the disturbance which causes the production of a dwarf egg is usually of an accidental or at least temporary nature. However, there are certain pathological conditions of the oviduct which result in the formation of a dwarf egg instead of a normal egg.

The 11 cases where dwarf egg production appeared to be related to a permanent disturbance of the physiology of the sex organs include all of cases where the bird produced more than three dwarf eggs, two that produced three, one that produced two, and four that produced only one dwarf egg. The production of a succession of dwarf eggs or of a long series of nesting records with one or two dwarf eggs should lead one to suspect a serious disturbance of the oviduct.

We will first consider dwarf-egg production which is not associated with a morphological abnormality of the sex organs and will then discuss the pathological cases.

## VIII.—THE RELATION OF DWARF-EGG PRODUCTION BY NORMAL BIRDS TO THE AGE OF THE BIRD AND TO THE POSITION OF THE EGG IN THE LITTER AND CLUTCH

### A.—AGE

Attention has already been called to the fact that while dwarf eggs may be produced at any season of the year the spring breeding season, the season for highest normal-egg production, is also the season for highest

dwarf-egg production, both in actual number of dwarf eggs and in the proportion of dwarf to normal eggs produced. It was, however, shown that the maximum dwarf-egg production (either absolute or relative) did not coincide with the maximum normal-egg production.

Earlier studies (5) have shown a decided relation between the age of the bird and the tendency to produce multiple-yolked eggs—that is, birds are more likely to produce double- or triple-yolked eggs before they are entirely mature than later in life. In this connection it seemed worth while to investigate a possible relation between dwarf-egg production and age.

There were 189 normal[1] birds which laid one or more dwarf eggs. These birds laid 205 dwarf eggs. The age of the bird at the time the dwarf egg was laid could be determined in 202 cases. The age frequency distribution is given below.

| Age in days. | Dwarf-egg frequency. | Age in days. | Dwarf-egg frequency. |
|---|---|---|---|
| 150–209 | 11 | 690–749 | 6 |
| 210–269 | 14 | 750–809 | 2 |
| 270–329 | 22 | 810–869 | 2 |
| 330–389 | 65 | 870–929 | 0 |
| 390–449 | 52 | 930–989 | 0 |
| 450–509 | 19 | 990–1,049 | 1 |
| 510–569 | 4 | 1,050–1,109 | 1 |
| 570–629 | 0 | | |
| 630–689 | 3 | | 202 |

The constants calculated from this frequency distribution are: Mean = 396.53 ± 6.43 days; standard deviation = 135.57 ± 4.55 days; and coefficient of variation 34.19 ± 1.27. These constants must not, however, be accepted as a description of the age variation. It has already been noted that a large proportion of the birds are disposed of at the end of their first laying year—that is, when they are 15 to 17 months of age. There were, therefore, many more chances for a bird to lay a dwarf egg during her first year than later in life. From data in hand it is not possible to decide whether or not a bird is more likely to lay a dwarf egg during the second or third year than during the pullet year. The flocks were not depleted, however, except by the normal small mortality from natural causes, until the end of the first laying year. It may be noted from the distribution that pullets are increasingly likely to lay dwarf eggs up to the time they are 1 year old and that the chances then decrease up to the end of the pullet year. The mean age for dwarf-egg production among pullets may be calculated from the above distribution as far as and including the 450–509-day group. This mean is 361.96 ± 3.75 days, approximately 1 year. It is apparent also that the second year maximum falls in the 690–749-day group—that is,

[1] That is, a complete study of their records, checked in many cases by post-mortem examinations, showed no abnormality.

when the bird is approximately 2 years old. Dwarf eggs are also produced by birds approximately 3 years old. From these data we see that dwarf egg production, unlike multiple-yolked-egg production, is not associated with immaturity of the bird, but that it is most likely to occur during the height of the breeding seasons in the successive years. These are, of course, the seasons of highest normal-egg production. In the case of a very few of the young birds and in an appreciable percentage of the old birds this is the only season in which the birds are in laying condition.

### B.—POSITION IN THE LITTER

There is a widespread popular belief that a dwarf egg marks the end of a laying period or litter. This belief has found frequent expression in the literature from an early period to the present day. König-Warthausen (7) summarizes the belief of Tiedemann (25) as follows: "Er hält die dotterlosen Zwergeier für 'Reste von in Eileiter abgesondertem Eiweiss und Kalkerde' nachdem durch Jahreszeit oder Alter das Legen zu Ende ist." To this, however, he adds his own observation, "dass solche Fehlgeburten vielfach bei erstlegendern Hühnern (in meiner Sammlung aus Marz, April, und Mai) stattfinden." Wright (28, p. 579), in his discussion of normal eggs, says: "Of the other kinds of abnormal eggs the very small ones only containing albumen usually occur at or near the end of a batch of eggs." That this relation of the occurrence of a dwarf egg to a particular position in the litter is still somewhat generally accepted is shown by two recent statements. Lewis (9) says that "extremely small eggs are common at the beginning and end of a laying period." The second statement referred to occurs in an unsigned article on "Xenia in fowls" in the Journal of Heredity (29) and is as follows:

> Experiments during recent years show that the eggs of any individual hen tend to become a little smaller as she approaches the end of her laying period, and the last one, it is generally believed, is likely to be a dwarf.

Since both dwarf eggs and broody hens are most common during the breeding season, it is not unnatural that a relationship between the two is assumed by poultrymen who do not trap-nest their birds. The use of the trap nest, however, soon dispels this illusion. Pearl, Surface, and Curtis (21) say that "the laying of one of these eggs is popularly supposed to mark the end of a laying period. This belief is without foundation in fact. They may be produced at any time." Warner and Kirkpatrick (26) some years later arrived at the same conclusion after a study of the data collected during two laying contests at Storrs. They summarize their data on this point as follows:

> It was found that only two eggs out of a total of 103 indicate a resting period after the production of a small egg. In every other case the small egg was found in an

almost uninterrupted series of normal eggs. This seems to prove conclusively that small eggs may be laid at any time during a hen's laying period and that most small eggs are laid while hens are at the height of production.

The data used in the present investigation confirm the main part of this statement—that is, dwarf eggs may be produced at any time during the laying period. Our figures do not show that they are less likely to be produced at either end of the period than during its midst, as the above authors seem to imply by their statement that "most small eggs are laid while hens are at the height of production." It is quite possible that they do not intend to make such an inference. Their records show that out of 103 eggs 7 were laid after a resting period of 14 to 25 days and 2 were followed by such a resting period. Our own records for normal birds which produced dwarf eggs and which completed the period of production during which the dwarf egg was laid show that out of 183 dwarf eggs 8 were first and 11 last eggs in their respective litters. A further analysis of our data on the position of the dwarf egg in the litter follows.

A few birds lay practically continuously from the beginning of laying until the first molt. Usually, however, there are well-defined laying periods which alternate with periods of nonproduction. The periods of production vary in extreme cases from two weeks to several months. In the present investigation any period of practically continuous laying, whatever its length, is considered a litter. In order to determine the relation of the production of a dwarf egg to its position in the litter, it is necessary to standardize the litter for the purpose of summarizing the data from the different cases. If the ordinal number of the day in the production period be divided by the whole number of days in the period, the resulting fraction will represent the position in the litter of an egg produced on that day. By this method the litter position of each dwarf egg produced by a normal bird which completed the litter was obtained. The frequency distribution for litter position of dwarf eggs is given below.

| Fraction of litter. | Dwarf-egg frequency. |
|---|---|
| 0 —0.099 | 26 |
| .100— .199 | 19 |
| .200— .299 | 10 |
| .300— .399 | 16 |
| .400— .499 | 13 |
| .500— .599 | 21 |
| .600— .699 | 24 |
| .700— .799 | 15 |
| .800— .899 | 10 |
| .900— .999 | 29 |
| | 183 |

Mean = $0.506 \pm 0.015$

Standard deviation = $0.307 \pm 0.011$

This distribution is shown graphically in figure 3. It does not show a tendency of a dwarf egg to be produced at any particular position in the litter—that is, the variation in the class frequencies are irregular. The dash line in the figure represents the mean class frequency; in other words, it represents graphically the frequency distribution for 183 observations evenly distributed among 10 classes. It is the ideal distribution of things equally likely to fall into any one of the 10 classes.[1] The question which concerns us is whether or not the actual distribution differs from this ideal distribution by an amount greater than we would expect if the differences are due entirely to errors in sampling.

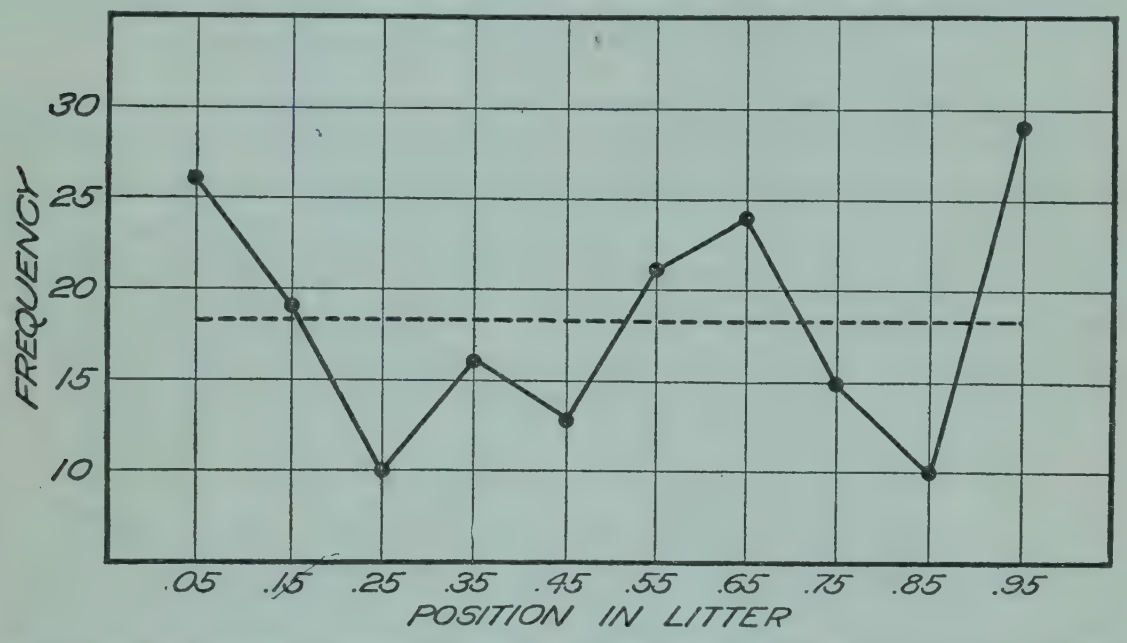


FIG. 3.—Diagram showing the number of dwarf eggs which occurred in each tenth of a litter. Dash line = the mean frequency.

A frequency curve of a given area is defined by its mean and the moments about this mean. The first four moments or the three constants, standard deviation, $\beta_1$ and $\beta_2$, derived from these moments define a curve sufficiently for practical purposes. Two frequency curves of equal area which differ significantly from each other will show a significant difference between one or more of the similar constants. These constants and also the mean, which gives the location of the curve in space, were computed for both the actual and the ideal distribution. These constants, with their probable errors, and the difference between the similar constants for the two curves, with the probable error of difference, are given in Table XXV.

[1] A general treatment of this "horizontal line" frequency curve, which is a special case of Pearson's Type II, will shortly be published elsewhere.

TABLE XXV.—*Mean standard deviation, $\beta_1$, $\beta_2$, and the difference between the similar constants for the two distributions for the actual frequency distribution of the position of dwarf eggs in the litter and for an ideal evenly distributed frequency of the same size* [a]

| Distribution. | Mean. | Standard deviation. | $\beta_1$ | $\beta_2$ |
|---|---|---|---|---|
| Actual | 0.506±.015 | 0.307±.011 | 0.003±.001 | 1.73±.05 |
| Ideal | .500±.014 | .287±.016 | 0 | 1.78±.06 |
| Difference with probable error of difference | .006±.021 | .020±.019 | .003 | .05±.08 |

[a] These constants are equal for any evenly distributed 10-class frequency with a class unit of 0.1, but the probable errors given in the table are calculated on the basis of 183 observations.

The last line of Table XXV shows that in no case does an essential constant for the actual curve differ from the similar constant for the ideal curve by an amount which is certainly significant—that is, the irregular fluctuations of the frequency curve for the litter position of dwarf eggs are not greater than the expected fluctuations of a random sample of the same size drawn from a population evenly distributed over the range. The present data, then, indicate that a dwarf egg is equally likely to occur at any time during a period of production.

### C.—POSITION IN THE CLUTCH

A fowl seldom lays on every day during a litter. The actual time between successive eggs depends on the rate of fecundity of the individual at the time. This rate differs greatly with the individual and with the season of the year. It also, in general, increases from the beginning of a litter to a maximum and then decreases toward the end of the period of reproduction (4, 19). Since fecundity finds its manifestation in discrete units (eggs), the result of a very low rate is expressed by the production of an egg on a day preceded and followed by one to several days on which no egg is produced. A common low fecundity rhythm results in the production of an egg on every second day. More usually an egg is produced somewhat later on each of two or more successive days, and then a day follows on which no egg is produced. The next egg is produced early on the following day. The litter is thus objectively broken into a series of daily eggs, which we may call "clutches," separated by one or more days on which no egg is produced. The size of a clutch varies from one egg to the extreme and unusual cases where a whole litter (sometimes of more than 40 eggs) is laid in a continuous daily series.

The general acceptance of the notion that a dwarf egg marks the end of a period of production suggests an investigation of the position of the dwarf egg within its clutch. In 197 of the cases where a normal bird produced a dwarf egg the bird completed the clutch to which the dwarf

egg belonged. Table XXVI gives for every size of clutch the frequency distribution of clutch position of dwarf eggs.

TABLE XXVI.—*Clutch-position frequency of the dwarf eggs for every size of clutch*

| Number of eggs in the clutch. | Ordinal number of the egg in the clutch. | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st. | 2d. | 3d. | 4th. | 5th. | 6th. | 7th. | 8th. | 9th. | 10th. | 11th. | 12th. | 13th. | 14th. | 15th. | Total. | Percentage. |
| 1 | 50 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 50 | 25.38 |
| 2 | 26 | 20 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 46 | 23.35 |
| 3 | 10 | 19 | 13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 42 | 21.32 |
| 4 | 5 | 5 | 7 | 7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 24 | 12.18 |
| 5 | 2 | 4 | 3 | 2 | 5 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 16 | 8.13 |
| 6 | 0 | 2 | 2 | 0 | 1 | 0 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 5 | 2.54 |
| 7 | 1 | 1 | 0 | 0 | 1 | 3 | 0 | ... | ... | ... | ... | ... | ... | ... | ... | 6 | 3.05 |
| 8 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | ... | ... | ... | ... | ... | ... | ... | 3 | 1.52 |
| 9 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | ... | ... | ... | ... | ... | ... | 2 | 1.01 |
| 11 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ... | ... | ... | ... | 1 | .51 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | 1.01 |
| Total | 95 | 51 | 26 | 10 | 8 | 3 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 197 | 100.00 |

This table (XXVI) shows that 50 dwarf eggs occurred as 1-egg clutches—that is, no egg was produced on either the preceding or the following day. Forty-six occurred in 2-egg clutches, the other egg being in each case a normal egg. Of these, twenty-six were the first, and twenty the second, of the two eggs. Similarly through the table we may compare the number of dwarf eggs produced in the successive positions in a clutch of any given size. The clutches in which dwarf eggs occurred varied in size from one to fifteen eggs. A study of this table shows no apparent uniform tendency for a dwarf egg to occur in any particular position in a clutch.

In order to summarize the data for the various-sized clutches, it is necessary to standardize the clutch. A clutch may be conceived as a line of definite length. This line may be divided into as many segments as there are eggs in the clutch. Each segment may be assigned a value equal to the fraction which the distance from the origin to the midpoint of the segment is of the whole length of the line. An egg, then, has a definite clutch-position value expressed as a fraction of the clutch. These values are comparable for all sizes of clutches. For example, the value assigned to the middle egg of any clutch which contains an odd number of eggs is 0.500. A table was calculated which gives the value for each clutch position in each size of clutch. By means of this table the clutch position for each dwarf egg can be determined in terms which are comparable for all cases of dwarf-egg production whatever the size of the clutch.

The clutch-position frequency for the occurrence of dwarf eggs is given below.

| Fraction of clutch. | Dwarf-egg frequency. |
|---|---|
| 0. —0. 199 | 19 |
| .200— .399 | 40 |
| .400— .599 | 25 |
| .600— .799 | 35 |
| .800— .999 | 28 |
| | 147 |

Mean $=0.518 \pm 0.015$

Standard deviation $=0.267 \pm 0.011$

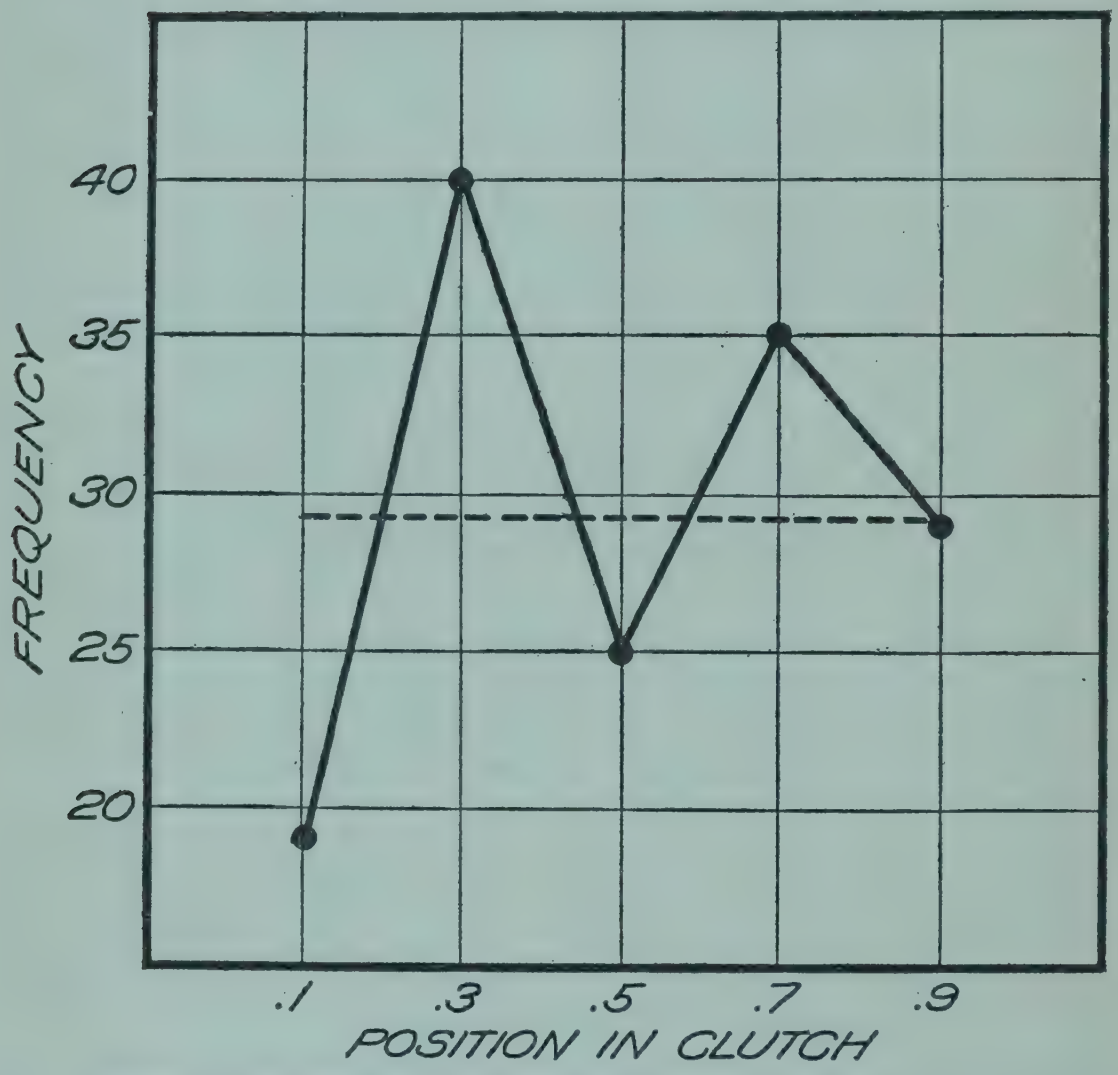


FIG. 4.—Diagram showing the number of dwarf eggs which occurred in each fifth of a litter. Dash line shows the mean frequency.

This distribution is shown graphically in figure 4.

There is no apparent relation of dwarf-egg production to any particular position in the clutch. The dash line in the figure, as in figure 3, represents an ideal uniform frequency for the same number of observations similarly grouped. In this case, as in the case of litter position, the actual frequency was tested against the ideal uniform frequency to determine whether or not the irregular fluctuations were greater than

would be expected from errors in sampling. The mean and the three constants which define frequency curves, standard deviation, $\beta_1$ and $\beta_2$, were calculated for the actual and the ideal distribution. It has already been pointed out that two distributions with the same number of observations which differ significantly will show a significant difference between the values for one or more of these similar constants. The constants for each distribution with the differences between the similar constants in the two curves are given in Table XXVII.

TABLE XXVII.—*Mean, standard deviation, $\beta_1$, and $\beta_2$, for the actual frequency distribution of the position of dwarf eggs in the clutch compared with the same constants for an equal even distribution with the same number of classes*

| Distribution. | Mean. | Standard deviation. | $\beta_1$ | $\beta_2$ |
|---|---|---|---|---|
| Actual..... | 0. 518±0. 015 | 0. 267±0. 011 | 0. 00005±0. 00003 | 1. 749±0. 054 |
| Ideal..... | . 500± . 016 | . 283± . 011 | 0 | 1. 700± . 054 |
| Difference with probable error of difference... | . 018± . 022 | . 016± . 016 | . 00005 | . 049±. 076 |

The last line of Table XXVII shows that not one of these constants for the actual distribution differs significantly from the similar constant for the ideal distribution—that is, the irregular fluctuations in clutch position of dwarf eggs are not greater than would be expected to occur from errors of sampling. The present data indicate, then, that a dwarf egg is equally likely to occur in any clutch position.

## IX.—PHYSIOLOGICAL CONDITIONS AND EFFECTIVE STIMULI WHICH LEAD TO DWARF-EGG PRODUCTION

It has been shown that dwarf eggs usually represent some temporary disturbance or some accident in the physiology of reproduction, since such eggs are preceded and followed by normal eggs. The disturbance is most likely to occur during the height of the breeding season, although it may happen at any time during the year. During any particular litter or clutch a dwarf egg is equally likely to occur at any time. Although the cause of dwarf-egg production is usually of a temporary character, there are cases where a bird lays only, or chiefly, dwarf eggs for some time. Other birds produce normal eggs for some time and then become habitual dwarf-egg producers. In the present section we will consider the nature of the disturbances, both temporary and permanent, which lead to the production of dwarf eggs.

Tiedemann (25) explained the origin of the dwarf eggs as the residue of albumen and shell secreted in the oviduct at the end of the laying. Wright (28) says that the occurrence of small abnormal eggs "need seldom

occasion anxiety. They usually occur at or near the end of a batch of eggs and merely show that the ovary is exhausting its supply of ova or yolks a little before the secreting parts of the oviduct are quite ready to suspend business." Lewis (8) explains dwarf-egg production, which he says is common at the beginning or end of a laying period, as "in part due to a diminution in the size, hence in the lessened secreting power of the oviduct." These views are untenable in the face of the facts cited above. Bonnet (2) says that such eggs mostly arise through pathological processes in the oviduct.

On the basis of unpublished data, Pearl, Surface, and Curtis (21, p. 176) made a statement of the factors which were probably involved in dwarf-egg production. The data on which this statement was based are included in the data used in the present investigations. The data then on hand indicated that three fundamental factors are concerned in dwarf-egg production. These are:

1. The bird must be in an active laying condition; the more pronounced the degree of physiological activity of the oviduct the more likely are these eggs to be produced.
2. There must be some foreign body, however minute, to serve as the stimulus which shall start the albumen glands secreting. This foreign body may be either a minute piece of hardened albumen, a bit of coagulated blood, a small piece of yolk which has escaped from a ruptured yolk, etc.
3. It seems likely, though this is a point not yet definitely settled, that ovulation—that is, the separation of a yolk from the ovary—must precede the secretion of albumen around the foreign body to form one of these eggs.

To a large extent the complete investigation confirms and extends these conclusions. The data which contribute to our knowledge of the physiology of dwarf-egg production are the complete egg records and the autopsy records of dwarf-egg producers.

### A.—EVIDENCE FROM THE EGG RECORDS AND AUTOPSY RECORDS OF DWARF-EGG PRODUCERS WITH ABNORMAL SEX ORGANS

It has already been noted that the egg records for 11 of the 200 known dwarf-egg producers showed that few or no normal eggs were produced after the dwarf egg or eggs. Such birds usually make nesting records, the dwarf egg occurring in a series of the nesting records. As an illustration, the egg record of case 1 is given in Table XXVIII.

From this record it may be seen that the bird was a heavy layer, producing 162 eggs up to May 28. After this she produced only one normal egg (on June 26). The nesting records occurring in clutches indicate that the ovary passed through its normal cycles. Four dwarf eggs were produced in a series of nesting records. The bird made her last nesting record on January 16. Twenty-four days later (February 9) she was killed for data. She was in a normal healthy condition and was very fat. The visceral organs were apparently all perfectly normal, except the oviduct. The ovary contained an enlarging series of yellow yolks, four

of which were more than 1 cm. in diameter. There were no visible discharged follicles. The bird was evidently approaching another cycle of egg production. The oviduct was nearly the size of an oviduct in a laying bird of the same body weight. The organ had but one abnormality. Six cm. from the mouth of the funnel were two constrictions, separated by about 1 cm. of duct, with the same diameter as the rest of the albumen region. The finger could be pushed through these constrictions. There was no pathological appearance in the duct wall at these points. It seems probable, however, that these constrictions prevented the passage of the normal egg, but allowed the passage of a smaller body, as the beginning of the dwarf egg. No yolk was found in any of the dwarf eggs produced by this bird. The nucleus in each of three cases was one or more small lumps of coagulated albumen. The dwarf egg produced on November 21 contained a small-stalked hard-shelled dwarf egg. The entire egg weighed only 11.1 gm. Neither the outer nor inner egg contained any yolk.

TABLE XXVIII.—*Egg record of case 1.*[a]

| Date. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | Totals. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sept. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | n | .. | .. | n | .. | .. | .. | .. | .. | n | .. | 1 | .. | 1 | 1 | .. | 3 | |
| Oct. | .. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | .. | 1 | 1 | 1 | n | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | .. | 27 | |
| Nov. | 1 | .. | 1 | .. | 1 | n | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | .. | 1 | .. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | .. | 25 | |
| Dec. | .. | .. | 1 | 1 | 1 | 1 | .. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | .. | 1 | 1 | 1 | 1 | 1 | 1 | .. | 1 | .. | 1 | .. | .. | .. | n | n | 1 | 21 | |
| Jan. | .. | 1 | 1 | 1 | .. | 1 | 1 | .. | 1 | 1 | .. | 1 | 1 | 1 | .. | 1 | .. | 1 | n | 1 | .. | 1 | .. | .. | 1 | 1 | .. | 1 | .. | 1 | .. | 18 | |
| Feb. | 1 | .. | .. | .. | 1 | .. | 1 | .. | 1 | .. | 1 | .. | 1 | n | 1 | .. | 1 | n | 1 | .. | 1 | .. | 1 | .. | n | .. | 1 | 1 | .. | .. | .. | 13 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 107 |
| Mar. | .. | 1 | .. | 1 | .. | 1 | .. | 1 | 1 | .. | 1 | 1 | .. | 1 | 1 | .. | 1 | 1 | 1 | .. | 1 | 1 | n | 1 | .. | 1 | 1 | 1 | n | 1 | .. | 19 | |
| Apr. | 1 | n | 1 | 1 | 1 | 1 | n | .. | 1 | 1 | 1 | n | 1 | .. | 1 | 1 | n | 1 | 1 | .. | 1 | 1 | 1 | 1 | .. | 1 | n | .. | .. | .. | .. | 18 | |
| May. | 1 | 1 | .. | 1 | 1 | 1 | 1 | 1 | 1 | n | 1 | .. | 1 | .. | 1 | 1 | n | .. | n | 1 | .. | 1 | 1 | n | 1 | 1 | .. | 1 | .. | .. | .. | 18 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 162 |
| June. | n | .. | .. | n | n | n | n | n | .. | n | n | n | n | n | n | n | .. | .. | n | n | n | n | n | n | n | 1 | n | .. | n | n | .. | 1 | |
| July. | n | n | n | 1— | n | n | n | n | n | n | n | n | n | n | n | n | n | n | n | n | .. | .. | n | n | n | n | n | n | n | n | 1— | |
| Aug. | n | n | n | n | n | n | .. | n | n | n | n | n | n | n | .. | n | .. | n | n | n | n | n | n | n | .. | .. | .. | .. | .. | .. | .. | 0 | |
| Sept. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | n | n | n | n | n | n | n | n | n | n | .. | n | n | n | n | n | .. | n | .. | n | .. | 0 | |
| Oct. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | |
| Nov. | .. | .. | .. | .. | .. | .. | .. | .. | .. | n | n | .. | n | n | n | n | .. | .. | n | .. | 1— | n | .. | 1— | .. | 1— | n | .. | n | n | .. | 3— | |
| Dec. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | n | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | |
| Jan. | n | .. | .. | n | n | n | .. | .. | .. | .. | .. | n | .. | n | .. | n | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | |
| Feb. | .. | .. | .. | .. | .. | .. | .. | .. | D | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | |

[a] "1" denotes a normal egg, "1–" a dwarf egg, "n" a visit to the trap nest but no egg, and "D" date killed for data.

Case 2 was that of another good layer which suffered a permanent disturbance, which hindered the production of normal eggs, but permitted dwarf-egg production. The case history of this bird follows. She began to lay on September 3, 1913. From this time until she stopped laying for the first molt, on October 5, 1914, she laid 218 eggs. On November 6, 1914, while the bird was still in nonlaying condition, three-fourths of her ovary was removed by a surgical operation. She began to lay again on December 29, 1914, and from this time until July 8, 1915, she produced 128 eggs. Three days later, July 11, she produced a dwarf egg. This was the first of a series of 7 dwarf eggs, the last of which was produced on July 23. Then followed an 8-day nonproductive period followed by a clutch of 3 normal eggs, on July 31 and Au-

gust 1 and 2. These were the last normal eggs produced. One more dwarf egg was produced on August 7. On August 29 the bird died of peritonitis.

At the autopsy a cylindrical dwarf egg was found in the oviduct. It was projecting from the isthmus into the shell gland. The egg had a shell which was thicker at the posterior end. Both the membrane and shell were incomplete at the anterior end. Several days before this bird died an egg similar to this one was found on the roosting boards of the pen in which she was kept. The funnel region of the oviduct was apparently normal. The glandular ridges were smeared over with what appeared to be albumen mixed with a small amount of yolk. At the anterior end of the albumen-secreting region the glandular ridges were very thick, and nodules of what appeared to be glandular tissue projected through the muscular layers. The region was somewhat contracted. Behind a narrow band of this tissue the duct was normal. In the body cavity free yolk was smeared over the intestines, and one yolk was walled off by peritoneum just below the ovary. Unfortunately no record was made of the contents of the egg found in the duct. Four of the eight dwarf eggs which were laid contained small drops or lumps of yolk. The other four were yolkless. All eight contained coagulation fibers which looked like normal chalazæ. The four yolkless ones contained no nucleus except these chalaza-like masses.

Apparently the ring of pathological tissue formed a partial constriction which hindered the passage of normal yolks. Yolks evidently entered the duct and were either extruded into the body cavity unbroken, or were broken and then entirely or mostly extruded. These yolks stimulated the secreting functions of the duct. In case all or most of the yolk was extruded the result was a dwarf egg. Three yolks evidently passed the obstruction unbroken and became the yolks of normal eggs.

Case 3 was that of a bird which was a fair producer during the early part of the season. She laid 150 eggs before July 1 and had always made some nesting records. The proportion of nesting records to eggs increased through the spring and early summer. In July there were as many nesting records as eggs. In August only two eggs were laid. The last of these, on August 27, was the last normal egg produced by this bird. From August 30 to October 16 the bird nested on every day except three. The only egg laid during this time was one dwarf, which was produced on September 16. The bird nested twice in November. There were two clutches of nesting records early in December. On December 13 the bird laid an egg which contained a normal yolk but which had a projection like a snail shell on the large end. The projection was formed of a membranous tube continuous with the egg membrane and filled with albumen. This tube was folded down onto the end of the other part of the egg and

was covered with a cap of shell. There was a distinct seam between the base of this cap and the shell which covered the rest of the egg, although they were continuous. Two days after this egg was laid the bird was killed, and an autopsy was performed. There was an egg in the oviduct just entering the isthmus. The lower end was covered with membrane. The upper end was prolonged into a string of albumen 5 or 6 mm. in diameter which extended 5 or 6 cm. up the duct. This egg then was similar to the egg laid two days previously in that it failed to round off normally at the anterior end. There was an abnormality of the oviduct which consisted in the presence of nodules of tissue in the glandular ridges of the funnel region. The nodules gave this lower portion of the funnel a quite abnormal appearance. Nodules were present in the peritoneum as well as in the oviduct. There were two large empty follicles on the ovary and a normal series of enlarging yolks, five of which were above 1 cm. in diameter. The other viscera were also normal. At the time of the autopsy the tumorous nodules in the lower funnel did not prevent the passage of yolks. The long series of nesting records at the time the dwarf egg was produced suggests that for a long period the pathological conditions of the duct may have prevented the passage of a normal yolk. The dwarf egg produced in the midst of this long series of nesting records contained some yolk wrapped in the chalazal fibers and some mixed with the thick albumen. The last normal egg had been laid 20 days before the dwarf egg. It seems therefore certain that the yolk in the dwarf egg was a part of a yolk which was broken either in the process of entering the duct or after it had entered. In the latter case the most of the yolk must have been extruded into the body cavity.

Another high producer which suffered a permanent disturbance which hindered normal-egg production was case 4. This bird produced 247 eggs during her first laying year. She had made occasional nesting records from the start, but the proportion of these to eggs increased markedly after July 1. There were, however, periods when the bird produced a litter of eggs without making nesting records. On February 13 of her record year the bird produced a dwarf egg. The egg which preceded this was a normal egg laid 13 days earlier. The dwarf egg contained chalaza-like coagulated albumen fibers, but no trace of yolk or other inclusion. Two days later the bird produced a normal egg. This was the last egg laid. Occasional nesting records followed. On May 5 (70 days after the last egg) the bird died and an autopsy was made. The ovary contained a normal series of enlarging yolks and four ruptured follicles. The body cavity contained a yellow fluid which was apparently a mixture of yolk and serum. A tumorous growth consisting of small solid tissue nodules was scattered all over the mesentery. A few nodules were present on the walls of the intestine. The upper half of the oviduct was badly diseased. The walls were thickened and hard. In places they were covered with large bunches of tumorous tissue.

This case again shows that a normal heavy-laying bird may develop a disease which affects the oviduct and prevents the passage of normal yolks, but which does not prevent the formation of yolks in the ovary. These yolks are ovulated into the body cavity. Since there was no yolk in the dwarf egg, it can not be proved that the egg formation was initiated by the entrance of a yolk which was later extruded. This may, however, have been the case. The occurrence of a normal egg only two days later shows that the ovary was in active condition. The immediate cessation of normal-egg production, the continued occasional occurrence of nesting records, and the condition of the ovary and oviduct at the autopsy strongly suggest that the passage through the duct was already considerably obstructed at the time the dwarf egg was produced.

The complete record of one more case (No. 5) is available. This bird did not begin to lay until November 13. She laid nearly continuously and made no nesting records until July 10. During this time (240 days) she produced 160 eggs. From July 10 to 23 the records show neither nesting nor eggs. This probably represents a normal period of nonproduction. No normal egg was produced by this bird after this period of nonproduction. On July 23 a dwarf egg was produced. This was followed by nesting records on the 24th and 27th. On the 31st another dwarf egg was produced. On August 3 and 4 the bird nested and on the 5th she produced a third dwarf egg. This was the last egg produced. From this time until the bird was killed (Sept. 2) nesting records continued to occur in series similar to the clutches of normal-egg production. We have no record or the contents of the dwarf egg produced on July 21. The eggs produced on July 23 and August 5 contained no yolk, but had as nuclei lumps of hardened albumen. The egg laid on August 5 was a dwarf egg which had a stalk attached to the large end. This stalk contained albumen and was covered with membrane and shell. To the lumps of albumen in this egg were attached long chalaza-like fibrous strings. One of these extended into the stalk. The autopsy record of this bird shows the ovary in a normal period of reproduction with a series of enlarging yolks, five of which were more than 1 cm. in diameter. There were four empty follicles visible. The anterior half of the oviduct was pathological. The walls were covered with a tumorous growth which appeared to be a proliferation of the muscular tissue. The outer layers of the walls of the intestine, portions of the oviduct ligament, and a small portion of the surface of the ovary contained small nodules of similar tissue. The body cavity contained a serous yellow liquid in which were lumps of yolk. The fact that the three dwarf eggs occurred between the production of the last normal egg and the complete cessation of egg production suggests that the disease may have gradually obstructed the passage through the duct. Whether or not the dwarf eggs were initiated by yolks which entered the duct and were later extruded can not be

decided, since they did not contain a trace of yolk. The continued occurrence of nesting records and the condition of the ovary at autopsy show that the reproductive cycles of the ovary were not interrupted. The dwarf eggs occurred during such a cycle.

The five cases of dwarf-egg producers cited above have several things in common: (1) Each bird was a normal, high-laying individual which became unable to produce normal eggs on account of a pathological condition of the oviduct. (2) In every case the part of the duct affected was the posterior end of the funnel, or the anterior end of the albumen-secreting region, or both. (3) The disturbance in each case was of a nature to constrict or prevent the normal expansion of the lumen of the duct. (4) In no case was the passage completely closed. (5) In each case there was convincing evidence that the ovary was in a normal reproductive cycle at the time the dwarf egg was produced.

Five of the sixteen [1] dwarf eggs produced by these birds contained as a nucleus a small quantity of yolk not inclosed in a vitelline membrane. This yolk was no doubt a part of a normal yolk, the rest of which was absorbed by the visceral peritoneum. Three of the five birds were absorbing yolk in this manner at the time of autopsy. The presence of a part of a yolk in the egg may have been due to any one of several causes. The three which seem most probable are the following:

1. A yolk may have been broken during its passage into the duct and only a part of it may have entered the duct.

2. A part of a yolk ovulated into the body cavity and, broken either before or after ovulation, may have been picked up by the funnel.

3. A normal yolk may have entered the duct and being unable to pass the pathological portion may have been broken and a part of it extruded into the body cavity. The remaining portion may have passed the obstruction, becoming the effective stimulus for the formation of the egg envelopes.

The effective stimulus in the case of the dwarf eggs which do not contain any yolk is difficult to ascertain. Some of these eggs contained what were apparently normal chalazæ. Most of them contained coagulated fibers which resembled the fibers of which chalazæ are formed. It is possible that in some or all of these cases a normal yolk has entered the duct, stimulated the upper duct to secrete chalazæ and some albumen, passed as far as the obstruction, and then been extruded, leaving behind sufficient chalazal material and albumen to furnish the mechanical stimulus necessary for the completion of the egg. Some of these eggs contained lumps of hardened albumen which may have arisen from albumen left in the duct or abnormally secreted. When the ovary is in a particular condition, such a mechanical stimulus may cause the secretion of the egg envelopes. It must be kept in mind, however, that a dwarf egg did not

[1] In two other cases the presence or absence of yolk was not recorded.

occur unless the ovary was actively producing yolks. In none of the above-mentioned cases was it impossible that a yolk had entered the duct and started the formation of the egg.

We have considered 5 of the 11 cases of dwarf-egg producers which were apparently permanently abnormal. Autopsies were not performed on the six other cases. The egg records for five of them (No. 6 to 10) resembled the egg records of the birds just discussed. No normal eggs were produced after the dwarf egg or eggs; also the birds made nesting records similar to egg records, indicating that the ovaries passed through normal reproductive cycles. The relation of the occurrence of dwarf eggs to normal eggs or nesting records was of a nature to show that they were produced only when the ovary was maturing yolks. Several of the dwarf eggs contained free yolk.

The record of the other bird (case 11) is worthy of special mention. This bird laid 17 dwarf eggs. These eggs were also produced when there was evidence that the ovary was in functional condition. The uniqueness of the case lies, first, in the unusual number of dwarf eggs, and second, in the fact that, although the number of dwarf eggs and nesting records and the proportion of these to normal eggs increased, some normal eggs were produced as long as there was any evidence that the bird's ovary was in laying condition. The egg record of this bird is given in Table XXIX.

TABLE XXIX.—*Egg record of case*[a]

| Date. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | Totals. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sept. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | n | n | .. | 1 | 1 | ... | n | ... | n | .. | 1 | .. | 1 | 1 | .. | 1 | .. | .. | 6 | |
| Oct. | 1 | ... | n | .. | 1 | 1 | .. | 1 | .. | n | .. | 1 | .. | n | .. | .. | 1 | .. | 1 | ... | 1 | ... | 1 | 1 | .. | 1 | .. | 1 | ... | n | .. | 12 | |
| Nov. | ... | n | ... | .. | 1— | .. | .. | 1 | .. | 1 | .. | ... | 1 | .. | 1 | .. | 1 | .. | n | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 6 | |
| Dec. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .. | 1 | n | .. | 1 | 1 | .. | 1 | 1 | ... | 1 | 1 | .. | 1 | .. | 1 | .. | 1 | ... | 1 | 1 | 12 | |
| Jan. | ... | 1 | ... | 1 | 1 | .. | .. | 1 | .. | 1 | .. | 1 | .. | 1 | .. | 1 | .. | 1 | 1 | ... | 1 | ... | 1 | .. | 1 | ... | .. | .. | ... | .. | .. | 13 | |
| Feb. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .. | ... | .. | ... | .. | ... | .. | .. | ... | ... | ... | ... | 1 | .. | .. | 1 | 1 | .. | 1 | .. | .. | 4 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | — | 53 |
| Mar. | 1 | ... | 1 | 1 | ... | 1 | .. | 1 | .. | n | .. | ... | 1 | ... | .. | 1 | 1 | .. | 1 | 1 | 1 | ... | 1 | 1 | 1 | ... | 1 | .. | 1 | .. | 1 | 17 | |
| Apr. | 1 | ... | 1 | 1 | ... | 1 | n | 1 | .. | 1 | .. | 1 | .. | 1 | .. | 1 | 1 | .. | 1— | ... | 1 | 1 | .. | 1 | 1 | ... | 1 | .. | 1 | 1 | .. | 18 | |
| May. | ... | 1 | ... | 1 | ... | 1 | .. | 1 | .. | 1— | .. | 1— | ... | ... | 1 | .. | n | .. | n | ... | 1— | n | .. | ... | .. | n | .. | .. | ... | n | .. | 8 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | — | 96 |
| June. | 1— | 1 | ... | n | ... | .. | 1 | ... | .. | 1— | 1 | ... | n | n | .. | 1 | n | n | n | 1— | ... | 1— | 1 | ... | .. | 1— | .. | .. | 1— | 1 | .. | 12 | |
| July. | ... | 1— | n | .. | n | n | n | ... | 1 | ... | 1 | n | 1 | ... | ... | ... | ... | n | ... | n | ... | ... | n | ... | ... | ... | n | ... | ... | ... | n | 4 | |
| Aug. | ... | 1— | ... | ... | n | n | .. | 1— | .. | ... | 1 | n | .. | 1 | .. | 1 | 1 | .. | 1 | n | ... | ... | ... | ... | ... | 1-1 | ... | ... | 1 | ... | .. | 10 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | — | 122 |
| Sept. | 1 | 1— | ... | n | ... | n | .. | n | .. | n | .. | n | .. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .. | 2 | |
| Oct. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .. | 0 | |
| Nov. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .. | 0 | |
| Dec. | ... | ... | 1— | ... | ... | .. | 1 | ... | ... | ... | ... | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .. | 3 | |
| Jan. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | |
| Feb. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | S | .. | .. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .. | .. | |

[a] "1" denotes a normal egg, "1—" a dwarf egg, "n" a visit to a trap but no egg, and "s" date sold alive.

A record was made of the contents of 16 of these dwarf eggs. Not one of these contained yolk, but all of them contained varying amounts of chalaza-like fibers, some resembling normal chalazæ. Thirteen contained no visible nuclei except the mass of coagulation fibers. One egg (laid on August 26) also contained a small lump of hardened albumen. The one laid on June 26 contained, beside the mass of chalazal fibers, a

small lump of tough membrane resembling shell membrane. The one laid on June 10 contained what appeared to be an empty yolk membrane. It will be shown later that a dwarf egg sometimes contains a ruptured yolk membrane from which most of the yolk has escaped, but this membrane contained no yolk. If it was a yolk membrane, all of the yolk had been squeezed out.

It is of some interest that this bird was a breeder, and the normal eggs laid between February 23 and April 22 were incubated. All but two were fertile, and 44 per cent hatched. It will also be noted that on August 26 both a dwarf egg and a normal egg were produced.

The dwarf egg and nesting records of this bird seem to indicate some disturbance of the morphology or physiology of the oviduct, which frequently but not always interfered with the entrance of a normal yolk or prevented its passage through the duct. Since this bird was sold alive, there is no record of the condition of the sex organs. As in other cases of dwarf eggs without yolk, it is impossible to tell whether the eggs were initiated by a yolk which entered the duct and was then extruded, or whether the fibers of chalazal material or other inclusions were efficient stimuli.

### B.—EVIDENCE FROM THE EGG RECORDS AND AUTOPSY RECORDS OF NORMAL DWARF-EGG PRODUCERS ON WHICH AUTOPSIES WERE PERFORMED

Attention has already been called to the fact that, while occasional cases occur where dwarf-egg production is due to a permanent disturbance of the reproductive apparatus, it is in general not associated with such a condition. In fact, a dwarf egg may occur at any time in a clutch or litter, the production of normal eggs continuing as if the dwarf had been a normal egg. In these cases the egg records give no hint as to the reason for the production of a dwarf egg. Our only data are obtained from the contents of the egg and the autopsy examination of the reproductive organs. Such autopsy records are available for 27 of the 189 dwarf-egg producers, which were apparently normal at the time of the production of the dwarf egg. In 4 cases a dwarf egg was found in the oviduct or body cavity at autopsy. Only one of these birds had previously laid a dwarf egg. In 3 cases the bird was killed a few hours after the dwarf egg was laid. Autopsies were made on 20 other cases from 9 to 508 days after a dwarf egg was laid. While the general or permanent morphological condition of the sex organs of a dwarf-egg producer is shown by each of these records, the temporary condition of the organs at the time a dwarf egg is produced is shown only by the cases on which autopsies were made while a dwarf egg was in the duct or within a few hours after such an egg was laid.

At the time of autopsy the sex organs of the birds which had produced a dwarf egg from 9 to 508 days before death were in every stage of repro-

ductive activity from strictly nonlaying to fully functional condition. Eighteen of the twenty showed reproductive organs which were in every respect normal for their functional condition. Each of these birds had produced a dwarf egg [1] in a regular series of normal eggs and had continued to produce normal eggs in regular cycles. These birds either made no nesting records, or a few such records were scattered among normal eggs, as is frequently the case with normal birds which have never produced dwarf eggs. Whether or not these occasional nesting records indicate ovulations into the body cavity has never been investigated. Observations made on a large series of autopsies on laying birds indicate that ovulation into the body cavity is not a very rare accident in birds with sex organs which are morphologically normal.

In each of the other two cases also the dwarf egg was produced in a regular series of normal eggs and normal-egg production continued in regular series for months (case 19, nine months, and case 20, five months) after the dwarf egg had been produced. Case 19 never made a nesting record until eight months after the dwarf egg had been laid. She then made two and laid a litter of 11 eggs. These were the last eggs produced. During the next three months she showed no evidence of reproductive activity (neither eggs nor nesting records). She began the last week of her life with a series of four nesting records on successive days. Three days after the last of these she died of peritonitis. At the autopsy the ovary contained a series of seven enlarged but absorbing yolks and two empty follicles which could be certainly identified. The body cavity contained free decomposing yolk. The upper part of the oviduct was filled with an egg concrement composed of successive layers of coagulated albumen formed around a small tumor which was attached by a narrow neck to a glandular ridge in the albumen-secreting portion of the duct. This tumor was about the size of a normal yolk.

Case 20 continued to lay normally for five months after the dwarf egg had been laid. Three nesting records were distributed separately among 100 normal eggs. The bird then appeared to pass through a normal nonproductive period of nine days. She then laid two eggs. A week later two clutches of nesting records occurred. During the next month she laid three eggs and made two nesting records. Occasional nesting records occurred during the next three months, but no more eggs were produced. The bird was then killed for data. At the autopsy the ovary contained a series of six enlarging yolks and three distinguishable follicles. The body cavity contained lumps of yolk. The funnel and the oviduct ligaments in the region of the funnel were pathological. They were red and fluted in appearance. The elongated lips of the funnel in the region of ligaments were fused together so that the opening of the funnel was no larger than the diameter of the tubular portion of the duct.

[1] In one case two and in another three at widely separated dates and each occurring in a series of normal eggs.

At the time of autopsy cases 19 and 20 showed a pathological condition of the oviduct which prevented the entrance of a normal yolk into the duct. The fact that these birds continued to be good layers for nine and five months, respectively, after the dwarf egg had been produced and then, by the cessation of normal-egg production and the occurrence of nesting records, showed a disturbance in their normal-egg production makes it seem probable that the pathological condition found at autopsy did not originate until some time after the dwarf egg had been produced. At least it is not safe to assert that a pathological condition of the duct existed at that time.

Of the seven cases of birds which either had a dwarf egg in the oviduct or body cavity at autopsy or had laid such an egg a few hours before death all had normal sex organs in fully functional condition. Each of these cases seems worthy of brief description. Case 21 was a late-hatched pullet which did not show any[1] reproductive activity until February. She then made a series of four nesting records and produced the dwarf egg as her first egg. The egg contained two small lumps of a dark, hard secretion and stringy albumen threads which looked like untwisted chalazæ. There was no yolk in this egg. The bird was killed a few hours after the egg was laid. She was in every way a normal healthy bird. The ovary contained a normal series of six enlarging yolks and four large and three small follicles. In the body cavity there were a few centimeters of a serous fluid containing yolk. In this fluid were found strings of tissue which may have been vitelline membrane. This pullet was then in full-laying condition; but for some reason not associated with an abnormality of the oviduct the yolks did not enter the duct, but were ovulated into the body cavity and absorbed. The origin of the stimulus which initiated the formation of the dwarf egg is not clear. While it is possible that all or part of a yolk entered the duct and was later extruded, there is no evidence for or against this view.

Case 22 was a normal pullet which had produced 48 normal eggs. She produced five normal eggs on successive days and on the sixth day produced the dwarf egg. The egg contained a lump or drop of yolk the size of a bean. The bird was killed a few hours after the egg was laid. The ovary contained a normal series of five enlarging yolks and four large and four small empty follicles. These follicles were all apparently normal and empty of yolk membranes. The body cavity contained a fluid which was partly yolk. In this case there can be no doubt that a normal yolk was ruptured either during ovulation or afterwards in the duct or body cavity and that a part entered or remained in the duct, forming the nucleus for the dwarf egg, while the rest was being absorbed by the visceral peritoneum.

[1] A lone nesting record occurred in December, but the bird may have accidentally gone into a nest.

Case 23 had been laying normally. The dwarf egg was the third egg in a clutch. This egg contained a peculiar nucleus. It appeared to be a yolk membrane constricted in the middle. One half of this membrane was filled with yolk and the other half with a clear liquid resembling thin albumen. The bird was killed a few hours after this egg had been produced. The sex organs were in normal active condition. There was no yolk in the body cavity. Evidently the abnormal yolk was extruded from a follicle which presented no abnormal appearance after the yolk was ovulated. Since this yolk was much smaller than a normal yolk, it is probable that it was formed in one of the smaller follicles.

Case 29 was killed by the other birds. She had laid a dwarf egg four months earlier and had continued to lay until six days before death. At autopsy a membrane-covered dwarf egg was found in the body cavity. It contained a small amount of very light-colored yolk. The albumen was greenish. The largest empty follicle on the ovary was not more than 5 mm. in diameter. There were a few shiny granules on the peritoneal surface which appeared to be remnants of absorbing yolk. Apparently the dwarf egg had remained in the oviduct or body cavity for several days, as the yolk it contained must have come from a follicle which was nearly absorbed. In this case also a part of a yolk seems to have been the stimulus which initiated the formation of the dwarf egg, while the rest of the yolk was absorbed from the body cavity.

Case 25 was a bird which had been presented by Dr. Edith M. Patch to the Maine Agricultural Experiment Station for dissection. This bird was kept at the Station plant for a few weeks. During this time she laid several normal eggs, but her trap-nest record was not kept. When she was killed for dissection, her sex organs were in a normal active condition. The ovary had a regular series of enlarging yolks and four empty follicles, two of which were nearly full size. The isthmus of the oviduct contained a normal membrane-shelled egg. A small dwarf egg was found in the shell gland. This egg contained coagulated fibers of albumen which resembled untwisted chalazæ. There was no yolk or nucleus other than the coagulation fibers. No yolk was found in the body cavity. If in this case the small egg had been initiated by a yolk which was returned to the body cavity and absorbed, the small egg must have been in the duct long enough for the absorption to have been completed. The size of the follicles on the ovary made this seem improbable. The origin of the chalaza-like bunch of coagulation fibers is not known. It seemed probable that these were the efficient initiating stimulus which started the secretion of the rest of the egg.

Case 26 died from some unknown cause. In the shell gland a dwarf egg was found. This egg contained as a nucleus a small lump of hardened secretion the size of a pinhead. The sex organs were in a normal

active condition except that the five yolks on the ovary were beginning to be absorbed. There were two large empty follicles. (The bird had not laid for five days before death.) The body cavity contained free yolk. This bird had also ovulated into the body cavity and was absorbing the yolks. Whether or not any of the yolk had entered the oviduct and initiated the secretion and then been expelled is not known. None of it remained in the egg.

Case 27 was found dead where she had hung herself in a feed rack. A dwarf egg was found in the shell gland. This egg is shown in Plate CXIII, figure 1. It contained two drops of yolk surrounded by albumen, egg membranes, and a thin layer of shell. The body cavity contained a yellow liquid which seemed to be a mixture of yolk and serum. The oviduct was in a normal active condition.

The ovary contained a series of enlarging yolks and ruptured follicles. From the largest one of the latter yolk was dripping. On examination it was found that the stigma or rupture line of this follicle was forked at the end. The follicle had ruptured only along the two short arms of this forked line. The yolk membrane was broken, but remained within the follicle. An examination of the follicles which contained the growing ova showed that three out of four of these had forked rupture lines. The follicles removed from this ovary are shown in Plate CXIII, figure 1. The last four (*c*, *d*, *e*, *f*) show the follicles containing complete ova. Follicles *c*, *d*, and *f* have forked stigmata, while *e* has a normal straight stigma. Follicle *b* is the one which contained the ruptured and nearly empty yolk membrane. It can be seen from the illustration that the straight part of the stigma is unbroken, while the forked part is open. In this case it seems clear that the incomplete rupture of the follicle resulted in the bursting of the yolk membrane. A part of the yolk entering the duct furnished the stimulus for the formation of the dwarf egg. The rest of the yolk was being absorbed by the visceral peritoneum.

### C.—EVIDENCE IN CASES WHERE A DWARF EGG FORMS A PART OF A COMPOUND OR A DOUBLE EGG

#### *a*.—COMPOUND EGG OF WHICH ONE PART IS A DWARF EGG

Recently an abnormal egg was produced by a bird in the Station flock, which gives additional evidence as to the physiological conditions and nature of the stimuli which may result in the production of a dwarf egg. The shell of this egg is shown in Plate CXIII, figure 2. This egg was compound, and the two parts were of quite unequal size. The component which filled the larger part of the shell contained a normal yolk in a normal membrane but there was a slight tear in this membrane, and free yolk was protruding from this tear. The hole which faced the small component egg was quite small, and little of the yolk had escaped.

This part of the egg had normal chalazæ and thick and thin albumen. The other part, which filled the small portion of the shell, contained a drop of free yolk surrounded by a thick albumen envelope, which was quite distinct from the albumen of the large part of the egg. No thin albumen was present in this part of the egg. The egg had been opened by cutting and lifting off an elliptical piece of the large part of the shell. When the egg was turned out through this opening, only the large part came out. It was then seen that an incomplete shell membrane separated the two components.

This egg is analogous to the type of double-yolked eggs where the doubleness is visible externally by a depressed ring around the shell, and where internally there is a fold of shell membrane projecting into the deepest part of the furrow. In such double-yolked eggs the thick albumens are entirely separate. It has been pointed out by Curtis (5) that such an egg must come about from the union of two eggs while the first egg is entering the isthmus, since the formation of the egg membrane is a discrete process taking place immediately when the egg passes the isthmus ring.[1]

The compound egg described above evidently represents the union of a dwarf and a nearly normal egg at this point in the duct. The point of peculiar interest is that the yolk for the two parts of the egg seems to have come from the same normal yolk. The fact that the small component is situated at the end which would have been the pointed end of the larger part had it formed a single egg suggests that the dwarf egg preceded the normal egg through the duct. It is conceivable that during ovulation the yolk membrane was slightly ruptured and that a drop of free yolk entered the duct ahead of the main body of the yolk. While this seems the most probable explanation of the phenomenon, the shape of the egg may have been modified by the presence of a dwarf egg following. In this case the yolk may have been ruptured either before or after ovulation and a drop left behind may have stimulated the formation of the dwarf egg.

The bird which produced this compound egg succumbed to roup four days after this egg was laid. She laid a normal egg the day before she died and at autopsy a normal, soft-shelled egg was found in the shell gland. The reproductive organs were in normal active condition.

Two other compound eggs where one component was a dwarf egg have been produced at the station plant. In neither of these cases was there any external evidence of doubling. The eggs were about as broad as the average egg of the individual, but were perceptibly longer (in one case 13 mm.), so that they appeared very long and narrow compared to the other eggs of the birds. There was also no folding in of the egg membrane

[1] The fact that when an egg is entering the isthmus as much and only as much of it as has passed in is covered with membrane was first noted by Coste in 1874, and has since been observed by many investigators, including the authors (15, p. 106).

between the two parts, and thin albumen surrounded both thick albumen envelopes, which were distinct. In both cases the dwarf egg was at the pointed end and the normal egg at the blunt, or air-cell, end. In both cases the membrane of the yolk in the normal egg was uninjured. In neither case was there any yolk in the dwarf egg. The only visible nucleus in each case was a mass of chalaza-like coagulated albumen fibers. In these cases also the dwarf egg seems to have preceded the normal egg down the oviduct. The normal egg apparently overtook the dwarf egg at the end of the albumen-secreting portion of the duct. The origin of the coagulation fibers, which apparently furnish the stimulus for the formation of the dwarf egg in these cases, is not known.

In one case the compound egg was produced by a pullet one month after she began to lay. During this month the bird had produced nine eggs and nested without laying on eight days. The bird nested without laying on the first, third, fourth, and fifth days before the abnormal egg was produced. The day following the abnormal egg she neither nested nor laid. On the next two days she laid normal eggs. From this time on the number of nesting records decreased and the number of eggs increased. This is the only abnormal egg ever produced by this bird. She continued to lay well until sold at the end of her pullet year.

In the other case the bird was about a year old. At the time the egg was produced she had been laying steadily for a month and a half. All the eggs had been normal. The bird had not laid on the day preceding the production of the compound egg. On the following day she produced a dwarf egg which contained a mass of chalaza-like coagulated albumen fibers, but no yolk. These two abnormal eggs were the first eggs in a clutch of five, the three others of which were normal. The bird continued to lay for 4½ months—that is, until the end of August—never again producing an abnormal egg. She was sold one week after she stopped laying.

*b*.—DOUBLE EGGS IN WHICH THE INCLOSED EGG, AND SOMETIMES ALSO THE INCLOSING EGG, WAS DWARF

A dwarf egg is sometimes inclosed within a normal egg, or may furnish the nucleus of a larger dwarf egg (10). The cases of this kind which have occurred at the Station plant will in the near future be described in connection with a discussion of double or inclosed eggs. So far as possible, the description of cases will be left to a future paper. It seems necessary to summarize them here. A dwarf egg may be returned up the duct and meeting a normal egg may be included with it in a common set of egg envelopes. Of more interest to the present investigation are the cases where a dwarf egg is inclosed in a larger dwarf egg.

One case where such an egg was produced by a bird with a constricted ring of tissue in the upper oviduct has already been cited. This egg was the first of a series of three dwarf eggs. (See Table XXVIII.) The

nucleus in each of the other cases was hardened secretion. The inclosed egg was a very small-stalked dwarf egg with a hard shell. There was no yolk in either the inner or outer egg.

An egg similar to the one just described but much larger (weight, 32 gm.) was produced by a 2½-year-old bird which had laid normally until the end of her second breeding season. She stopped laying about the middle of June and showed no evidence of reproductive activity until the middle of August. She then began making nesting records. On the 25th she produced the double dwarf egg. A week later she was sold. The inclosed egg was a hard-shelled, stalked, dwarf egg which weighed 7 gm. The end of the stalk was open. This egg contained a mass of chalazal fibers and thin albumen. The long axis of the inclosed egg lay in the long axis of the inclosing egg. Coagulated albumen fibers like untwisted chalazæ were attached to both ends of the egg. The mass at the closed end of the inner egg contained a small cluster of yolk granules and a small lump of hardened secretion. The outer egg had both thick and thin albumen, normal egg membrane, and hard shell.

Four other cases have occurred at the Station plant where a very small dwarf egg has been the nucleus for a larger dwarf egg. In none of these cases was there any yolk in the outer egg, although in two of them there was a small amount of yolk in the inner egg. In each case the dwarf egg was covered by an egg membrane without shell. Each of the outer eggs had normal egg membranes and shell. In three cases there were bunches of coagulated albumen fibers resembling chalazæ attached to the poles of the inclosed dwarf egg. In each case the bird producing the egg was a normal heavy-laying bird and the egg occurred in a normal clutch of from two to five eggs. In each case the double egg was the only abnormal egg ever produced by the bird.

It thus seems that in normal birds in active laying condition a dwarf egg may be forced up the duct and may furnish the stimulus for the formation of a set of egg envelopes in which it becomes inclosed.

### D.—EVIDENCE FROM EGG RECORDS AND EGG CONTENTS

It has been shown above that in cases of dwarf-egg producers on which autopsies were made, both normal and abnormal birds produced dwarf eggs only when the ovary was in active condition. All cases on which autopsies have been made with a dwarf egg in the oviduct, or within a few hours after a dwarf egg was laid, showed large empty follicles. Every case but one showed also that a yolk had been ovulated at almost precisely the time the secretion of the egg envelopes of the dwarf egg began. In the other case the ovary contained a series of absorbing follicles, two of which were very large, indicating that both had been discharged within two or three days at most. One of these had furnished

the yolk present in the normal egg found in the isthmus. Since the egg record of the bird is not available, it is impossible to say whether the yolk discharged from the second large follicle had been contained in a normal egg laid within a day or two before death, or whether it had been absorbed with great rapidity from the body cavity. It has been shown by Pearl and Curtis (6, 16) that "yolks and partly or fully formed eggs may be absorbed rapidly and in large numbers from the peritoneal surface." Previous observations, however, would not lead us to expect that within the normal period of the formation of an egg in the duct the absorption of a yolk would be so complete that no trace of it could be found. It seems probable that the sex organs remained in a condition capable of response to a stimulus for egg production for a few hours after ovulation. The presence of two large follicles, however, shows that in this case also the sex organs were in the extreme of active condition.

In case an autopsy was not performed upon a bird which produced a dwarf egg the morphological condition and the physiological state of the sex organs at the time the dwarf egg was laid can be judged reasonably accurately by the egg record. In all cases not discussed under the section on abnormal physiological conditions associated with dwarf-egg production the dwarf egg was produced within a litter all the other eggs of which were normal. As already shown, the dwarf egg took any position in the clutch and litter. In all cases there was abundant evidence from the egg record that the sex organs were in active condition and were capable of producing normal eggs.

In the center of the thick albumen of every dwarf egg examined was found some firmer material. In a number of cases this firmer nucleus was simply a few coagulated threads of albumen which resembled the threads of a normal chalaza. Sometimes the mass of threads has the appearance of a normal chalaza, but more often it is an irregular mass of untwisted threads. Such a mass of threads, or one, rarely two, more or less perfect chalaza, is present in nearly all the dwarf eggs. Frequently it is accompanied by one or more small slightly reddish lumps which appear to be hardened albumen, or by small blood clots, or more frequently still by a drop or more of yolk. It has already been stated that more than half of all the dwarf eggs collected contained some yolk not in a yolk membrane. In these cases the yolk is frequently surrounded by a membrane-like layer of coagulated albumen fibers resembling a chalazal membrane. In many cases nearly normal chalazæ are attached. The contents of such an egg is shown in Plate CXIII, figure 3. In most of these cases there is no normal yolk membrane in the egg, but in a few cases the dwarf egg contained a ruptured normal yolk membrane from which most of the yolk had escaped. Beside these, each of a number of dwarf eggs contained a small yolk without a germ disk but inclosed in a complete vitelline membrane.

Table XXX gives the number and percentage of each kind of dwarf eggs classified as to the nature of the contained nucleus.

TABLE XXX.—*Dwarf eggs classified according to the nature of the contained nucleus*

| Nature of nucleus. | Number of dwarf eggs. | Percentage of dwarf eggs. | Subtotals of percentages. |
|---|---|---|---|
| Drop of yolk, no yolk membrane | 141 | 51.46 | ..... |
| Broken yolk membrane with some yolk | 10 | 3.65 | 55.11 |
| Small complete yolk | 27 | 9.85 | 64.96 |
| Chalazal threads with or without lumps of coagulated albumen or blood clots | 96 | 35.04 | 100.00 |
| Total | 274 | 100.00 | ..... |

From Table XXX we see that 55.11 per cent of all the dwarf eggs opened contained a portion of a yolk, and 3.65 per cent contained a broken yolk membrane. This fact, in connection with the autopsy records already discussed for birds killed while a dwarf egg was in the duct or immediately after one was laid, indicates that in at least 55 per cent of the cases the immediate stimulus to the active duct was a part of an egg yolk, the rest of which was absorbed from the visceral peritoneum. In case 27, discussed on page 1027, the vitelline membrane of the yolk which furnished the stimulus was still within its ovarian follicle, although part of the yolk was in the dwarf egg in the shell gland and most of the rest in the body cavity. In this case the yolk was broken during ovulation, and only a part of it entered the duct. In the other case it is impossible to tell whether the yolk was broken during or after ovulation. It is possible either that the yolk was ovulated into the body cavity and subsequently broken and a part taken up by the duct; or on the other hand, it may have entered the duct and later been broken and a large part of it expelled.

Parker (10) described an ovum in ovo where the inclosed egg was yolkless and the inclosing egg contained a little "yolk substance." He believed that this "yolk substance" was the remnant of a normal yolk which had been ruptured and most of which had escaped. This suggested to him the question, "Is it possible that the yolkless condition of the inclosed egg is also due to the loss of its yolk?" However, he believes the evidence convincing "that albumen can be formed by the oviduct without the presence of yolk."

In 9.85 per cent of the dwarf eggs the stimulus to the active duct was an abnormally small yolk which for some unknown reason was produced and ovulated by the ovary. These cases apparently differ from normal egg production only quantitatively—that is, in the size of the stimulating yolk.

It is seen from Table XXX that 64.96 per cent of all the dwarf eggs produced were apparently initiated by the presence of yolk in the duct.

The presence of almost normal chalazæ in a few of the eggs without yolk suggests that a yolk may sometimes enter the duct, stimulate secretion of chalazæ, and then be extruded, leaving behind enough chalazæ and albumen to furnish the necessary stimulation for the completion of the egg.

## X.—RELATION OF DWARF-EGG PRODUCTION TO OTHER OBSERVED PHENOMENA OF EGG PRODUCTION WHICH OCCUR IN NATURE OR HAVE BEEN EXPERIMENTALLY PRODUCED AND THE CONTRIBUTION OF THIS STUDY TO OUR KNOWLEDGE OF THE NORMAL PHYSIOLOGY OF EGG PRODUCTION

It has already been noted that five of the six birds on which autopsies had been performed while an egg was in the oviduct or immediately after one was laid were absorbing yolk through the visceral peritoneum. In three cases the dwarf egg also contained yolk. In two of the other cases, however, no yolk was found in the dwarf egg, although the body cavity contained yolk. This suggested, first, that ovulation or a specific condition of the sex organs immediately accompanying it was the essential stimulus for the secretion of the egg envelopes by the duct; or, second, that such a specific condition being present, the secretion of the egg envelopes was stimulated by the small lump of hardened albumen, which in these cases seemed to be the nucleus of the dwarf egg; or, third, that a yolk had entered and then been expelled from the duct.

That neither ovulation nor any condition of the sex organs associated with it is alone sufficient to cause the formation of a dwarf egg is certain. Birds known to have ovulated into the body cavity for a long time, due either to a morphological, physiological (6), or surgical (16) disturbance, which prevented the yolk from entering the duct but did not otherwise disturb the mechanism, did not produce dwarf eggs. Some stimulus other than the condition of the sex organs is necessary to start the secreting activity of the duct. In normal eggs and in dwarf eggs with yolk this stimulus (mechanical or chemical) is furnished by the yolk.

The fact that all dwarf eggs without yolks contain some nucleus firmer than normal albumen, together with the fact that in one case where the bird had a dwarf egg with such a nucleus in the shell gland at autopsy no yolk was found in the body cavity, suggests that when the sex organs are maturing and ovulating successive yolks from the ovary a mechanical stimulus may initiate the secretion of the egg envelopes.

Experiments performed by Tarchanoff (24) and Weidenfeld (27) have shown that a complete set of egg envelopes may be formed around an artificial yolk. Tarchanoff used an amber bead and Weidenfeld used an artificial yolk of wood or rubber. The authors, using a glass marble or an artificial yolk of agar, have confirmed this result. The experiments

have been referred to by one of the authors (5), but have not been described in detail. One of these eggs is shown in Plate CXIII, figure 4, *a*. The agar artificial yolk which formed the nucleus of this egg is shown in *b* of the same figure. This artificial yolk, which weighed 4.32 gm., was inserted through a slit in the middle of the albumen-secreting region and pushed posterior to the slit. The duct was tied on each side of the slit. The morning after the operation the membrane-shelled egg, which weighed 14.06 gm., was found on the floor of the cage.

In another successful case a glass marble coated with vaseline was inserted into the funnel, and the funnel was then closed by sewing the lips together. On the day following the operation the bird laid a hard-shelled egg which weighed 36.17 gm. This egg contained a small lump of vaseline as a nucleus. Six days after the operation the bird died. At autopsy the marble was found caught in the thread that sewed the mouth of the funnel. In this case it was impossible for a yolk to enter the duct, since the funnel lips were sewed together. The stimulation must have come from the marble or the vaseline.

Tarchanoff (24) notes that he obtained this result in only 1 out of 11 cases. The authors obtained a perfect result in only 2 out of 12 trials. Three other results were partially positive. In one case the bird was killed 24 hours after the operation and the agar yolk was found in the upper isthmus covered with a thin layer of thick albumen. In two other cases, where the birds succumbed to postoperative peritonitis, the artificial yolk, surrounded by a thin layer of coagulated albumen, was found in the duct at autopsy. In the other seven cases the artificial yolk was either laid without egg envelopes or was found naked in the duct at autopsy. All the birds used in these experiments were in active laying condition at the time of the operation. Two to three weeks after the operation autopsies were performed on five of the seven birds giving negative results. In two of these the sex organs were in the state to be expected in a bird which had stopped laying two or three weeks previously and was not approaching a new laying period. In the three other cases the sex organs were in functional condition, but no empty follicles were found on the ovary. We have noted elsewhere (16) that "a bird is usually not in laying condition for some time after any serious abdominal operation involving prolonged anesthesia and considerable surgical shock." Sellheim (23) notes that after removal of the oviduct the ovary at first shrinks; but since it comes again into functional condition, he believes that the postoperative shrinking is due to the severe operation. It seems that in the negative and partly positive cases described above the general physiological disturbance due to the operation may have lowered the general tone of the organism, or possibly the specific tone of the reproductive apparatus, to a point where the duct was unable to respond to stimulation in its normal manner.

The results show conclusively that in a certain stage of activity the oviduct responds to a mechanical stimulus by the secretion of the egg envelopes.

The fact that in a bird approaching a period of laying the oviduct enlarges as the yolk enlarges has long been recognized. In a bird which has not laid for two or three months and is not preparing for another production period the sex organs are in strictly nonfunctional condition. The ovarian eggs are scarcely larger than a pinpoint. The oviduct is a small, almost straight thin-walled tube, weighing from 2 to 3 per cent of its weight when in functional condition. As the ovary approaches laying condition, the oviduct enlarges. When the first group of oocytes start on their final growth period, the increase in the size of the duct is perceptible. By the time the first yolk is mature, the oviduct is also normally in functional condition. That this correlation is entirely due to the ovary is shown by the fact that the removal of the oviduct has no influence on the development or functional activity of the ovary (23, 16). Normally the oviduct is in functional condition only while the ovary is maturing yolks. The correlation is now commonly attributed to the internal secretion of the ovary. Bartelmez (1) working on pigeons states that "interstitial cells of the ovary show much greater signs of activity in functioning ovaries than do those in ovaries of birds that have not laid for a long time." A fact cited by Pearl and Curtis (16) indicates that the connection is not nervous, or at least that it is not conveyed to the oviduct through the nerves. This fact is that after the removal of a large part of the oviduct any part not removed passes through growth and cyclic changes associated with the periods of ovarian yolk production, exactly as though the duct were intact. Observations made in connection with other researches have shown that enlargement of the oviduct is not necessarily connected with yolk formation, although this is the normal relation. The two classes of exceptions that have been noted are: First, certain hermaphrodite fowls have been observed (14) that have ovaries largely made up of stroma rich in connective tissue and containing no large follicles, and yet these birds had oviducts from one-half to three-fourths the size of a functional duct; and, second, birds with certain types of ovarian tumors, but without enlarging yolks, have been observed to have nearly functional-sized ducts.

These facts taken together indicate that the functional condition of the oviduct depends upon some substance formed in the ovary, usually at the time yolks are maturing, but in certain pathological cases at other times also. This substance is probably an internal secretion carried by the blood, since the ovary can cause the enlargement to functional size of a small piece of oviduct the normal nervous connections of which have been destroyed. The fact that dwarf eggs are produced only when the bird is maturing and ovulating yolks and the fact that more than 50

per cent of the trials to induce egg formation around artificial yolks were failures suggest that the sex organs must be and must remain in absolute functional condition until the egg is completed.

Loeb (9) showed that the mammalian uterus responds to a mechanical stimulus by the formation of the maternal placenta during a definite period after ovulation. He finds that during this period the uterus is sensitized by the internal secretion of the corpus luteum. We may conceive that the specific state of the oviduct in the fowl which renders it capable of responding to mechanical stimulation, be it yolk or foreign body, is associated with some quantitative or qualitative difference in the internal secretion of the ovary. While from the data given above it is possible that it is due to some postovulation change in the ovary, this seems improbable. We know that in many and probably in most cases in normal-egg production the duct responds to the first yolk of a series ovulated. This response occurs immediately after ovulation—that is, there is not sufficient time for a change in the internal secretion of the ovary occurring at or after ovulation to affect the state of the duct.

An observation made some time ago also has a bearing on this point. A bird which had laid three days earlier was selected for an abdominal operation. She was accidentally killed with an overdose of ether just after the incision was made. The oviduct did not contain an egg, but the funnel was in active motion when first observed. It responded quickly and sharply when stimulated by pinching with the forceps. The albumen region also responded to this stimulus by strong peristaltic movements. A 10-cm. piece from the albumen-secreting region of this very active duct was cut open lengthwise and spread out flat with the glandular surface exposed in a warm damp chamber moistened with salt solution. Small bits of cork were scattered on the surface in order to study ciliary motion. The ciliary activity continued for 1½ hours. At the end of this time it was noted that a very thin film of albumen was visible on the surface of the mucosa. In this case an isolated piece of oviduct responded to mechanical stimulation by the secretion of a very small amount of albumen. This duct had not been sensitized by an immediately preceding ovulation. The last ovulation had taken place four days before the bird was killed. The active movements of the funnel when the body cavity was opened suggested that an ovulation was about to take place. Either the duct had remained in a condition capable of a secretory response for four days or it had again come into such a condition with the maturing of another yolk.

The above-described experimental work and the observations on the conditions under which dwarf eggs are produced indicates that mechanical stimulation of the oviduct results in the formation of egg envelopes only under a particular condition of the duct which seems to be associated with the maturing of yolks by the ovary. The sensitization of duct, if this is the proper explanation of the phenomena observed,

apparently precedes ovulation. Further work is necessary, however, to determine the factors involved in the specific condition of the duct which causes it to respond to stimulation by the secretion of the egg envelopes.

It would seem from the above considerations that the presence in a completely functional oviduct of a small solid or semisolid substance capable of presenting a mechanical stimulation may cause the production of a dwarf egg. Nearly two-thirds of the dwarf eggs, however, are known to be initiated either by abnormally small yolks or by parts of broken yolks. Their production may be associated with an abnormal condition of the ovary or with pathological conditions of the duct, but even in these cases the result was due not to the abnormality per se but to the fact that this abnormality prevented the entrance of a normal yolk or obstructed its passage through the duct.

The mechanical stimulus need not begin at the funnel in order to be effective to the parts of the duct lower down. In Tarchanoff's case (24) and in one of our own cases of perfect egg formation around an artificial yolk, the yolk was inserted into the duct through a slit in the albumen portion, the duct being tied off above this point. Pearl and Surface (18) showed that a mechanical stimulation (in this case feces introduced by anastomosing the intestine to the side of the uterus) caused the formation of shell by the uterus.

The mechanical stimulation is of local character—that is, it is not transmitted down the duct for any measurable distance below the point where it is applied. Pearl and Curtis (16) have shown that "the stimulation of the advancing egg is necessary for the discharge of the secretion of the duct, since a duct closed at any level functions only to the point where the passage is interrupted." In the cases of dwarf-egg producers with pathological ducts the abnormality of the duct was in each case of a nature to constrict but not close the lumen of the duct. Several eggs produced by these birds contained lumps of yolk, indicating that the nucleus of the egg had passed the constricted portion.

## SUMMARY

(1) During the eight years from February 1, 1908, to February 1, 1916, 298 dwarf eggs are known to have been produced at the poultry plant of the Maine Experiment Station.

(2) During the two years of maximum dwarf-egg production the ratio of dwarf eggs to normal eggs was 1 dwarf egg to 1,158 normal eggs.

(3) Dwarf eggs are of two distinct types in respect to shape: First, the prolate-spheroidal type, and second, the cylindrical type.

(4) Dwarf eggs of the prolate-spheroidal type are much more frequently produced than the cylindrical type. In fact 95.4 per cent of the dwarf eggs studied were prolate spheroids.

(5) Dwarf eggs may also be classified according to the absence of yolk or its presence either as a small yolk in a yolk membrane or as free yolk.

(6) Of the 274 dwarf eggs opened 35.03 per cent were yolkless and 64.96 per cent, or nearly two-thirds, contained yolk. The yolk was inclosed in membrane in only 9.85 per cent of the dwarf eggs opened, while free yolk was present in 55.11 per cent of these eggs.

(7) Dwarf eggs with small yolks, while distinctly smaller than normal eggs, are significantly larger than dwarf eggs with little or no yolk.

(8) A comparison of the relative size of the several groups of dwarf eggs, normal eggs, double-yolked and triple-yolked eggs furnish a continuous line of evidence that the amount of albumen secreted depends to a large extent at least upon the degree of immediate stimulation due to the amount of yolk present.

(9) Although the evidence available is not sufficient for a positive statement, the shape of the cylindrical egg is probably due to the long form of the stimulating nucleus.

(10) Dwarf eggs with small yolks have indices which are higher than those for normal eggs and lower than those for other prolate-spheroidal dwarf eggs. This difference in index in the three groups is the reverse of their difference in size.

(11) This negative correlation between the shape, index, and size extends the evidence from former researches that the smaller the egg the broader it is in proportion to its length.

(12) Two factors may be involved in producing this negative correlation between shape index and size: First, the area of the glandular mucosa under stimulation at any one time must be related to the size, particularly the length, of the stimulating nucleus (yolk drop, normal yolk, or two or three yolks in tandem). Second, the oviduct, which is a tube with elastic walls, will offer more resistance to the passage of a large than a small body, and therefore when the plastic egg is forced through it by peristalsis it will exert a greater elongating pressure upon a large than a small egg.

(13) Dwarf eggs of each class are exceedingly variable when compared to normal eggs. This greater variation occurs in all the physical characters measured—that is, length, breadth, shape index, egg weight, yolk weight, shell weight, and possibly albumen weight.

(14) Dwarf eggs with small yolk resemble normal eggs in degree of variability as well as in size and shape more nearly than do other classes of dwarf eggs.

(15) The several size characters show different degrees of variation. They may be arranged from most to least variable as follows: Egg weight, length, and breadth. This arrangement is the same for dwarf and normal eggs.

(16) It is probable that the variation in yolk weight compared to the variation in the other egg parts and to the whole egg is greater in dwarf eggs with small yolks than in normal eggs.

(17) The interrelation of the size and shape characters in prolate-spheroidal [1] dwarf eggs of each class is as follows:

*a.* Length and breadth, length and weight, and breadth and weight are significantly highly correlated in eggs of each group.

*b.* Index and weight are negatively correlated. The correlation is significant for dwarf eggs with little or no yolk.

*c.* In dwarf eggs with small yolks, yolk weight is highly correlated both with egg weight and with albumen weight.

The physiological significance of these correlations is discussed.

(18) During the last eight years 5.15 per cent of all the birds kept at the Maine Station plant are known to have produced at least one dwarf egg.

(19) Both the actual dwarf-egg production and the number of dwarf eggs per 1,000 eggs is lowest during the winter months. It increases through the spring, reaching a maximum in the early summer.

(20) In general the season of high normal-egg production is also the season for high dwarf-egg production both actual and relative to normal-egg production. The maximum of dwarf-egg production, however, occurs later in the season than the maximum normal-egg production.

(21) The production of a dwarf egg is usually an isolated phenomenon occurring only once or twice during the life of a bird. Only 3.5 per cent of the birds which produced one or more dwarf eggs produced more than two.

(22) A study of all the egg records and the available autopsy records for birds which produced one or more dwarf eggs shows that in most cases the disturbance which caused the production of the dwarf egg was of temporary character and was not correlated with a morphological disturbance of the sex organs.

(23) Eleven of the two hundred dwarf-egg producers, however, showed evidence that a permanent disturbance had occurred. In these cases few or no normal eggs were produced after the dwarf egg or eggs, although nesting records indicate that the ovary passed through normal reproductive cycles.

(24) Autopsies were made on five of these cases, and all of them showed some pathological condition of the oviduct which interfered with the passage of the egg, but did not entirely close the duct.

(25) In normal birds dwarf-egg production is most likely to occur during the height of the breeding season. It is not associated with immaturity of the sex organs.

[1] The same relations apparently also hold for cylindrical dwarf eggs, but the number observed was too small to determine the degree of relationship.

(26) The popular notion that a dwarf egg marks the end of a period of production is without foundation. A dwarf egg is equally likely to occur at any time during a clutch or litter.

(27) A dwarf egg may be overtaken by a normal egg and form one of the components of a compound egg similar to a double-yolked egg except that one part is a dwarf egg.

(28) A dwarf egg after it has received its membrane or its membrane and shell may be returned up the duct and be included in the succeeding normal egg, or it may act as the stimulus for the formation of a larger inclosing dwarf egg.

(29) Dwarf eggs are produced only when the ovary is in the absolutely active condition associated with the maturing of yolks. This is true whether the bird has a normal or pathological oviduct.

(30) When the sex organs are in this condition, a mechanical stimulation of the oviduct by an artificial yolk may result in the formation of a complete set of egg envelopes.

(31) The mechanical stimulation need not begin at the funnel in order to be effective to the parts lower down.

(32) The mechanical stimulation is local in its effect—that is, it is not transmitted down the duct any distance below the point to which it is applied.

(33) Dwarf eggs may be and probably often are produced by the stimulation of an active duct by some material particle which is not yolk.

(34) At least 65 per cent of the dwarf eggs studied, however, were initiated by an abnormal small yolk or by a part of a normal yolk. Certainly in some and probably in all the latter cases the rest of the yolk was absorbed by the visceral peritoneum.

(35) Neither the absolute time relation between ovulation and the ability of the duct to respond to mechanical stimulation nor the nature of the connection between the state of the ovary and the duct is certainly known.

(36) It is suggested that the oviduct may be sensitized by some change in the internal secretion of the ovary associated with the maturation of yolks.

(37) It is also pointed out that if this is the case the change in the secretion probably precedes ovulation.

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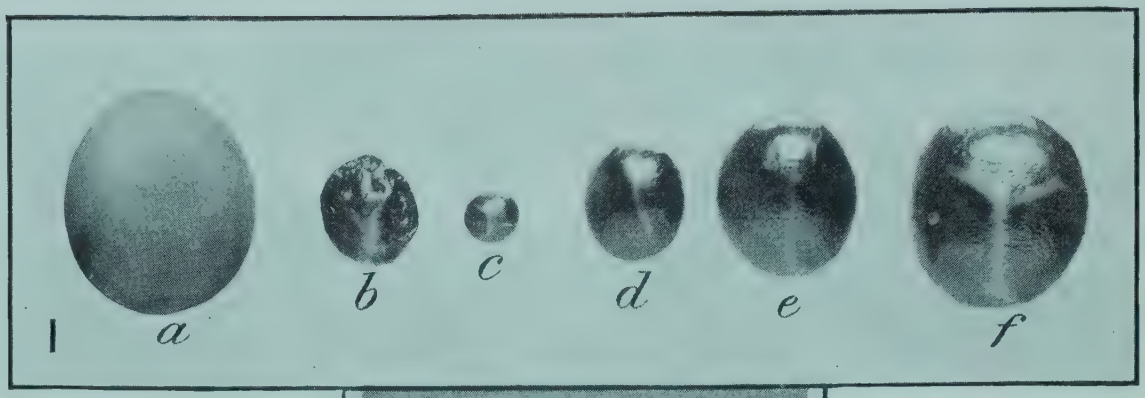
## PLATE CXII

A collection of dwarf eggs with a normal egg in the center of the group. × 2/3.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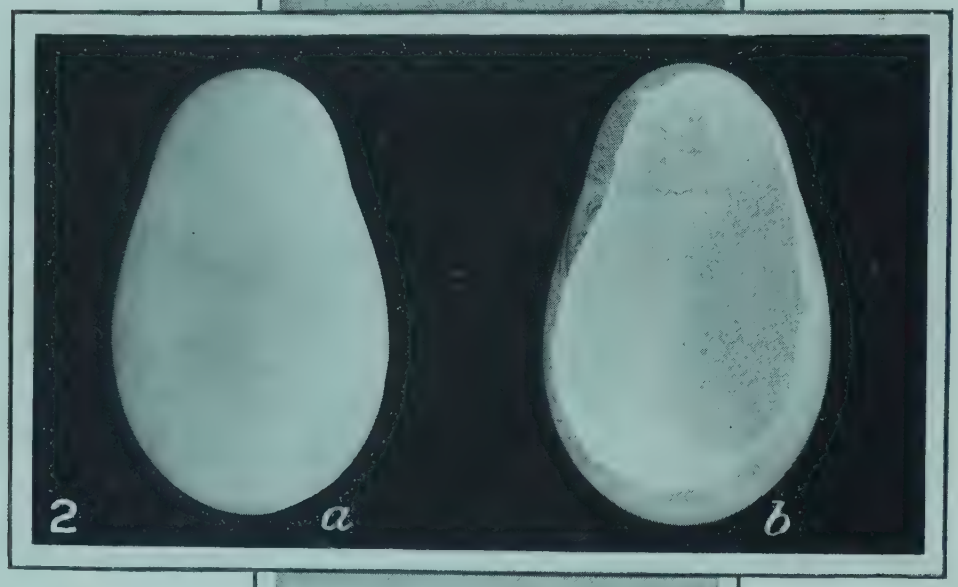
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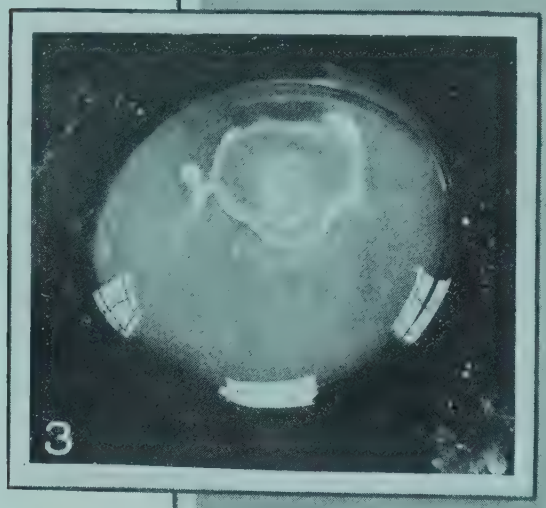
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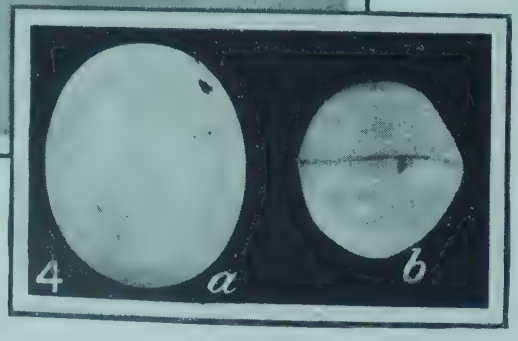
4
a
b

## PLATE CXIII

Fig. 1.—Ovarian follicles (*b–f*) and the dwarf egg *a* from case 27. Follicle *e* has a normal straight stigma. Follicles *b*, *c*, *d*, and *f* have stigmata which are forked at the end. The forked portion of the stigma of *b* has ruptured; the yolk membrane is broken and most of the yolk has escaped. Part of the escaped yolk was in the body cavity and part formed the nucleus of the dwarf egg *a*. × 2/3.

Fig. 2.—Shell of a compound egg which was composed of two albumen masses partly separated at the level of the seam in the shell by an incomplete egg membrane. The larger component contained a normal yolk with a slight puncture in the yolk membrane. The smaller one contained a drop of yolk which apparently came from the yolk in the other part. *a*, Outside view of shell; *b*, inside view. × 2/3.

Fig. 3.—Dwarf egg containing a mass of yolk not in a yolk membrane, but separated from the albumen by a membrane-like layer of chalazal threads. Note nearly normal chalazæ. × 2/3.

Fig. 4.—Dwarf egg formed around an artificial yolk of agar which was inserted into the oviduct. *a*, Complete egg; *b*, agar yolk. × 2/3.

# α-CROTONIC ACID, A SOIL CONSTITUENT

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*Biochemists, Soil-Fertility Investigations, Bureau of Plant Industry*

In a preliminary examination of a sample of Susquehanna fine sandy loam soil from Texas, which was made in October, 1915, by Dr. E. C. Shorey, who was at that time connected with the Office of Soil-Fertility Investigations, an unsaturated organic acid was isolated. In a subsequent examination of the same soil by the writers this compound was again isolated, and its identity with α-crotonic acid has now been established.

The soil was taken from an infertile spot in a field near Marshall, Tex. The infertile spots, which are devoid of all vegetation, have been observed for three years in this locality, and the area of these spots is gradually increasing.

The soil in this district is described as a Susquehanna fine sandy loam, from 8 to 18 inches deep, with an average of about 14 inches (8).[1] The subsoil is a stiff clay of a red color or red mottled with yellow and gray extending to a depth of several feet. The color of the soil is prevailingly gray, but for a few inches above the subsoil it has a reddish cast. Because of the impervious nature of the subsoil, the drainage is very poor, and special methods of soil management, with the object of producing better drainage, have been recommended and to a limited extent practiced. This soil is deficient in lime or other basic material and is very poorly drained. It has also been found to have a high reducing power and a rather low oxidizing power. It therefore seems to present optimum conditions for the formation and accumulation of organic acids.

In the isolation of α-crotonic acid an alkaline extract was obtained by treating the soil with an aqueous 2 per cent sodium-hydroxid solution for 24 hours at room temperature. The extract was made slightly acid with sulphuric acid and filtered. The acid filtrate was then extracted with ether and the ether extract was evaporated to about 200 c. c. and shaken up with a concentrated solution of sodium bisulphite to remove aldehydes or other substances which combine with this reagent.

The bisulphite solution was drawn off and extracted several times with fresh ether. All of the ether extracts were then combined and slowly evaporated to a brown sirup in a small crystallizing dish. At this point the dish was covered with a watch glass containing ether and maintained at a low temperature on a steam bath. A white crystalline solid gradually sublimed on the watch glass. The sublimed substance was dried between filter paper and recrystallized from petroleum ether.

[1] Reference is made by number to "Literature cited," p. 1045.

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fk

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G—94

The substance was further purified by subliming several times at a low temperature and was dried over anhydrous calcium chlorid.

The properties of the substance thus obtained were found to be identical with those of $\alpha$-crotonic acid. The purified soil substance melts at 72° C., while $\alpha$-crotonic acid melts at 72°. A mixture of Kahlbaum's chemically pure $\alpha$-crotonic acid (further purified by sublimation) and the soil compound melted at 72°.[1]

The purified soil compound is soluble in water, ether, alcohol, and slightly soluble in cold and more soluble in hot petroleum ether. It has a sharp odor somewhat similar to that of butyric acid, although much milder. It readily reduces potassium permanganate in a cold alkaline solution. In a cold aqueous solution it decolorizes bromin instantaneously, but does not decolorize bromin in carbon tetrachlorid. With ferric chlorid it gives an orange color on the spot plate. In aqueous solution it does not reduce gold chlorid in the cold.

A determination of the neutralization equivalent (molecular weight) gave the following results: 45.3 mgm. of the soil compound required 10.43 c. c. of *N/10* sodium hydroxid (NaOH) for complete neutralization with phenolphthalein as the indicator.

The neutralization equivalent was found to be equal to 86.8.

The neutralization equivalent calculated for crotonic acid ($CH_3CH$:-$CH.COOH$) is 86.05.

The soil substance sublimes readily at room temperature, which is in accord with the observation made by Bulk (1).

These reactions and tests on the soil compound and synthetic $\alpha$-crotonic acid were carried out simultaneously and were found to be identical in every case. The crystalline forms were also found to be the same. Figures 1 and 2 of Plate CXIV show the form and similarity of the crystals obtained in the first stage of sublimation. During the process of sublimation the crystals grow into large irregular plates or leaflets.

Ninety-four mgm. of the acid were obtained from 50 pounds of soil. This quantity would correspond approximately to 16 pounds per acre. It is obvious from the very unusual properties of this substance that a considerable amount would be lost in the processes of isolation and purification, and the actual amount present in the soil would be much greater than 16 pounds per acre, which is therefore a minimal value.

The $\alpha$- and $\beta$-crotonic acids are unsaturated and have the formula $CH_3CH$:$CH.COOH$. These acids are typical examples of compounds which exhibit geometrical isomerism. Their structures have been dwelt

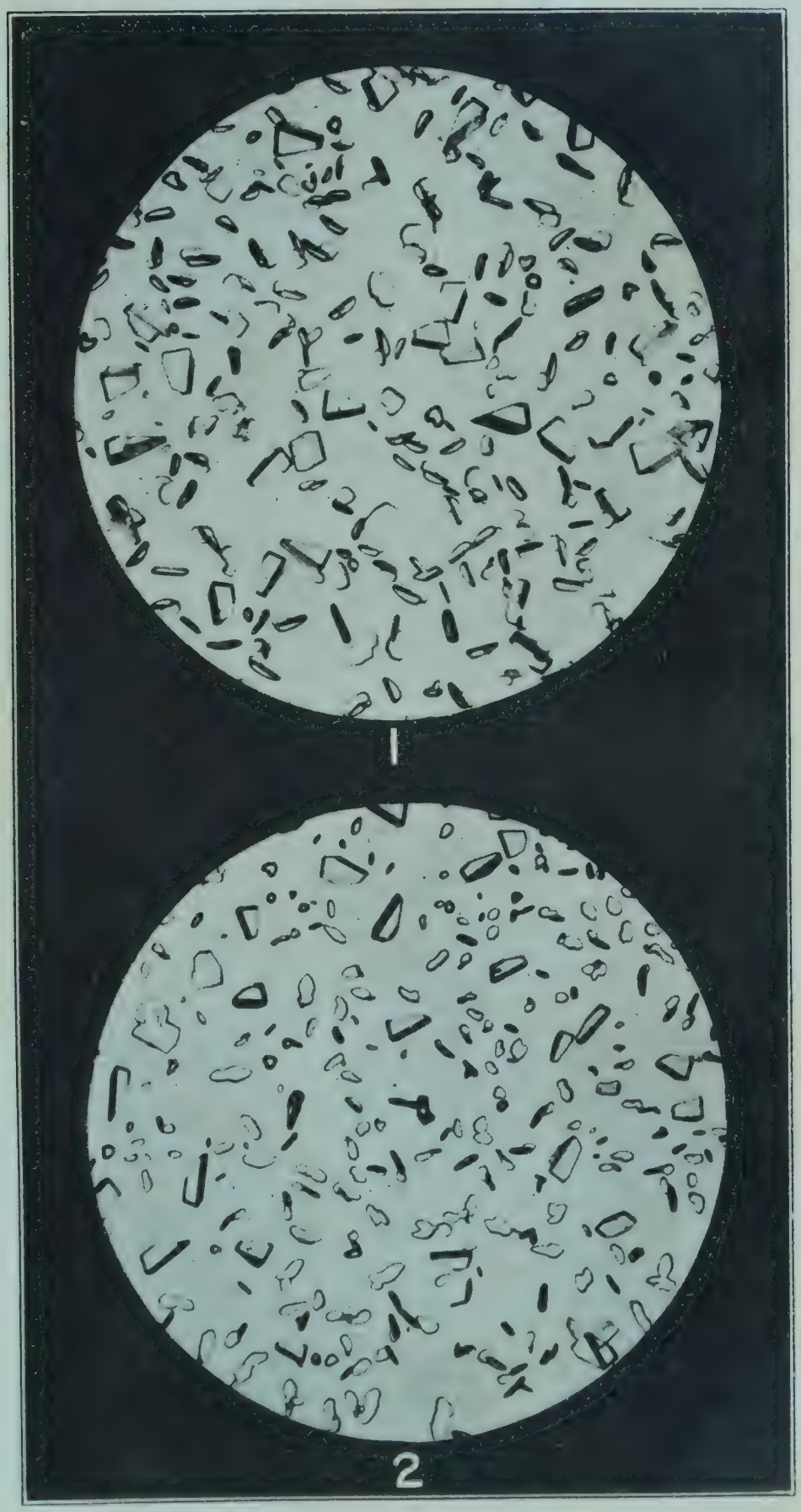
[1] In all cases a slight softening or sintering at 69° to 70° was observed, which may be due to the presence of traces of $\beta$-crotonic acid. Morrell and Hanson (4, p. 1522) have shown that $\alpha$-crotonic acid, when heated above its melting point, is partially converted into $\beta$-crotonic acid in amounts varying with the temperature. This study indicated the advisability of subliming $\alpha$-crotonic acid at a low temperature in purifying it in our work. In order to prevent the loss of material by sublimation, the melting points were made in sealed tubes which were completely submerged.

upon by numerous investigators (7) and are represented by the following formulæ:

$CH_3.CH$ $\quad\quad$ $CH_3.CH$
$\quad\quad\quad

## PLATE CXIV

Fig. 1.—α-Crotonic acid from soil. × 210.
Fig. 2.—Synthetic α-crotonic acid. × 210.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